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CORRIGENDA IN VOL. XXI

p. 316, lines 29 and 30: for "sheet" read "five."

p. 325, description of Plate XVII: for "white skin" read "heterozygous green, *yy(g)*."

p. 398, line 8 from bottom of page: for "218 BR" read "218 Brr."

FRITILLARIA MELEAGRIS: CHIASMATA- FORMATION AND DISTRIBUTION.

BY THE LATE W. C. F. NEWTON AND C. D. DARLINGTON.

(*John Innes Horticultural Institution, Merton.*)

(With Nine Text-figures.)

It is obvious from a cursory examination of the pollen mother-cell division of *Fritillaria Meleagris* that the chromosome behaviour is of an exceptional type. The late W. C. F. Newton referred to this briefly (1927). He said that "in *Fritillaria* there is never more than one point at which all four chromatids touch and this agrees in every instance with the point of attachment. The result is of course that no figure more complicated than the cross is formed in *Fritillaria*." This, as he remarks, "suggests a comparison with *Stethophyma grossum* of Janssens" (1924) but is otherwise scarcely to be paralleled¹. Newton realised the significance of this observation for the study of meiosis, and with a view to elucidating the whole phenomenon he had made the smear preparations which form the subject of the present study.

The mitotic chromosomes of *Fritillaria Meleagris* (Fig. 1)² closely resemble the complements of *F. imperialis*, *F. lanceolata* and *F. lutea* (cf. Taylor, 1926). They are probably identical in form with those of *F. latifolia* (which may be regarded as a Persian variety) illustrated earlier (Darlington (1929 b), Text-fig. 68 a). The somatic number of each of these species is 24, consisting of ten pairs with sub-terminal attachment constriction and two pairs with approximately median ones. In *Fritillaria Meleagris* six pairs of chromosomes have the minor segment reduced to roundness at metaphase, while four pairs have a slightly larger minor segment. One of the former types has the secondary distal constriction³ found very generally in the genus.

¹ Meurman (1929) in his exceptionally critical examination of chromosome forms in *Aucuba* has shown in three chromosome types (III, IV and V) that an interstitial chiasma always coincides with a secondary constriction, while in the other chromosome types the chiasmata are all terminal. In this case it would seem that a chiasma movement in terminalisation has been arrested by the constriction.

² Sections were cut at 30 μ and stained with gentian violet.

³ It is convenient to speak relatively to the attachment constriction.

The normal parasynapsis of a diploid occurs in the prophase of the pollen mother-cell divisions of *Fritillaria Meleagris*, that is to say homologous chromosomes associate probably throughout their length.



Fig. 1. Somatic metaphase of *Fritillaria Meleagris* from a root tip. Plan to show proximal¹ ends of chromosomes. $2n=24$. ($\times 2800$)

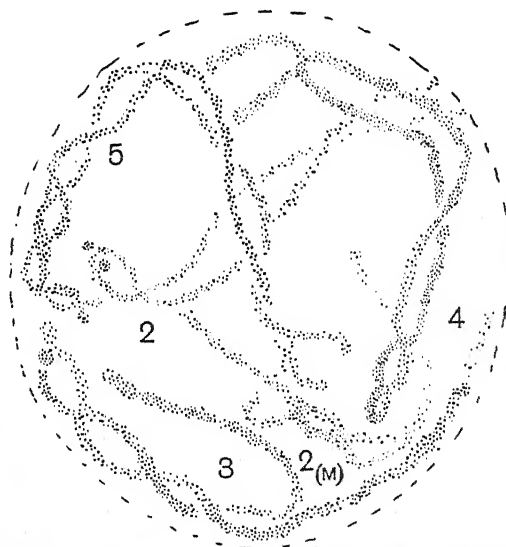


Fig. 2. Diplotene: four bivalent chromosomes with terminal constriction. The number of chiasmata is marked in each. ($\times 2800$)

At diplotene the four chromatids fall apart in pairs, making loops which meet so quickly that no exceptional features can be detected in the process. The result is the formation of chiasmata at various points on

¹ It is convenient to speak relatively to the attachment constriction.

the chromosomes where loops of different association meet, and the chromatids therefore change partners. These chiasmata, however, are not distributed at random along the chromosome (as has been described in every known case except *Stethophyma*) but are concentrated in the neighbourhood of the attachment constriction (Fig. 2). Their relationship to the attachment, as such, is unmistakable, for medianly constricted chromo-

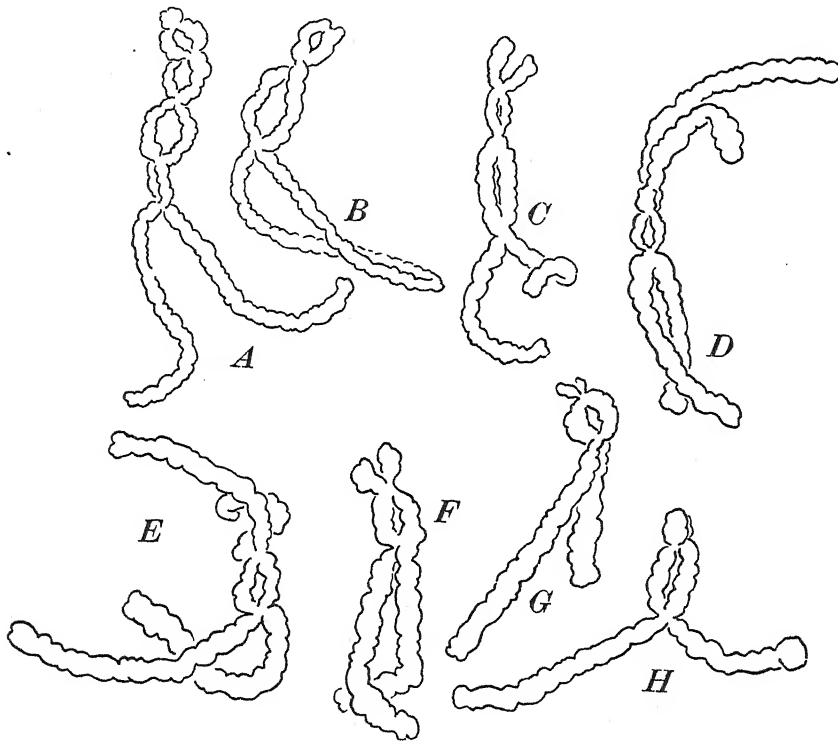


Fig. 3. Late diplotene. Eight bivalent chromosomes: *a*, *b*, *c*, *f*, *g* and *h* with terminal constrictions, *d* and *e* with median constrictions. Two to five chiasmata. ($\times 2800$)

somes (not found in *Stethophyma*) have in consequence a characteristic appearance. Nearly always the attachment is marked by a loop with a single chiasma on either side of it (Fig. 2M). Two or three chiasmata near one end characterise the subterminally constricted chromosomes. We are thus in the position of being able to distinguish chromosome types at diplotene; this is unexampled except in regard to the sex chromosomes of certain animals. Fig. 3 shows some of the characteristic structures observed at this stage. Between diplotene and diakinesis, as

the chromosomes contract, the successive loops come to dispose themselves at right angles.

In general, where several chiasmata occur in one pair, they lie closer together than in any comparable material we have studied where chiasmata arise at random along the chromosome. In other words there is, relative to the length of the chromosome, a shorter distance than usual between chiasmata. It follows therefore that the appearance in the contracted state at metaphase should be comparable to that found in smaller chromosomes. This is the case. It is only with difficulty that the chiasmata can be detected in most bivalents at diakinesis or at metaphase. In fact, without a knowledge of the principles of chromosome pairing or of the earlier stages one would scarcely be led to enquire further. A close study shows however that here, as in plants and animals with smaller chromosomes where the existence of chiasmata has usually been ignored, the superficial appearance is merely the result of extreme condensation of the loops formed at diplotene (Fig. 4).

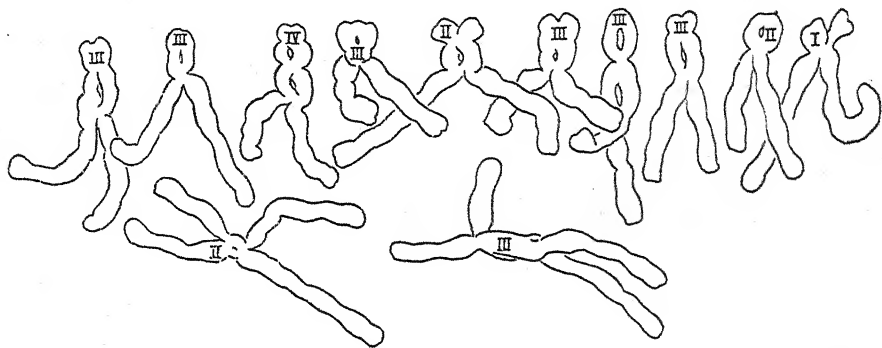


Fig. 4. Complete chromosome set at diakinesis. Each bivalent is marked with the number of its chiasmata. ($\times 2800$)

The resemblance of the regions near the attachment constriction to the whole chromosomes of maize for example (Longley (1927), Randolph (1927)) is remarkable. The absence in maize of the long free distal elements so decisive in *Fritillaria* must prevent any clear analysis of its bivalents, but their analogous appearance is none the less significant of an analogous structure. At metaphase a certain uniformity is imposed by the peculiarly constant distribution of the chiasmata, so that the chromosomes—to use Newton's non-committal phrase—usually appear to touch at one point only (Figs. 5 and 6). The exceptions are all the more striking, for occasionally a chiasma may be formed at, or close to, the end of a chromosome distal

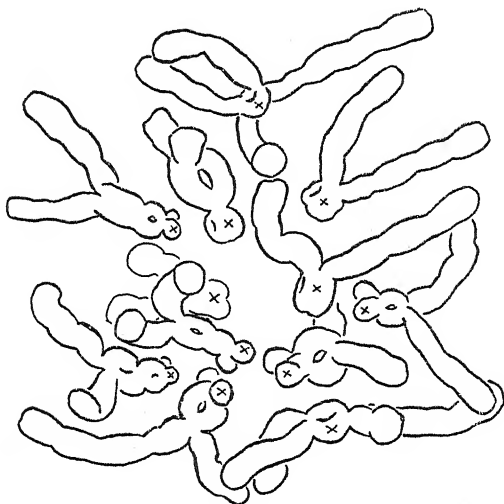


Fig. 5. Metaphase of the first division in polar view. In each chromosome the position of the attachment constriction is marked with a cross. The loop at the attachment is always in the axis of the spindle. The medianly constricted chromosomes are at 12 o'clock and 5 o'clock. Cf. Table I. ($\times 2800$)



Fig. 6. Metaphase of the first division. Side view. Cf. Table I. ($\times 2800$)

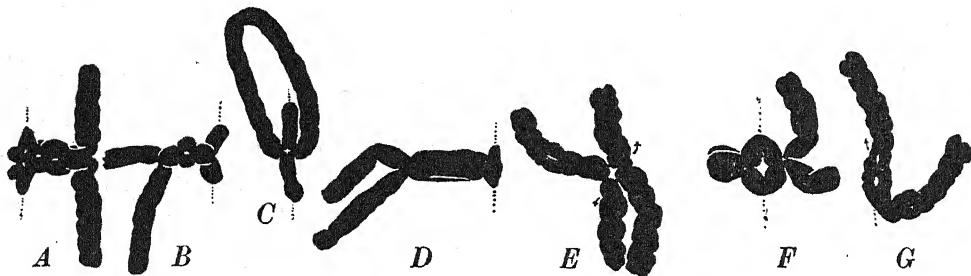


Fig. 7. Bivalents from metaphase of the first division in side view, showing exceptional formation of chiasmata. *a*, five chiasmata; *b*, four chiasmata; *c*, two chiasmata, one terminal (very rare); *d*, three chiasmata, as in *c*, an exceptionally large loop; *e*, one chiasma only in a medianly constricted chromosome; *f*, two chiasmata unusually distant from the constriction; *g*, one chiasma on the minor arm and none on the major arm of a sub-terminally constricted chromosome. ($\times 2800$). Note: *b*, *c* and *e* have no chiasma on the minor arm.

to the attachment. Such chiasmata have been observed at all various stages (Figs. 2 and 7 c).

Fragments of various sizes have been seen at metaphase. These have evidently arisen as in *Tulipa*, *Hyacinthus* (Newton and Darlington, 1929) and *Tradescantia* (Darlington, 1929 b) at prophase. Fragments have been found in the somatic complement of *F. imperialis* (Darlington, 1929 b), and it is worth recalling that the differences in the chromosome complement of the 24-chromosome *Fritillaria* species and the 18-chromosome *F. ruthenica* indicate a fusion in the one or a splitting in the other near the attachment constriction (Darlington, 1929 c), i.e. in the region of greatest chiasma frequency in *F. Meleagris*.

At anaphase polar views give a clearer idea of the relations of the chromatids to one another¹. Observations of this stage (Fig. 8) confirm

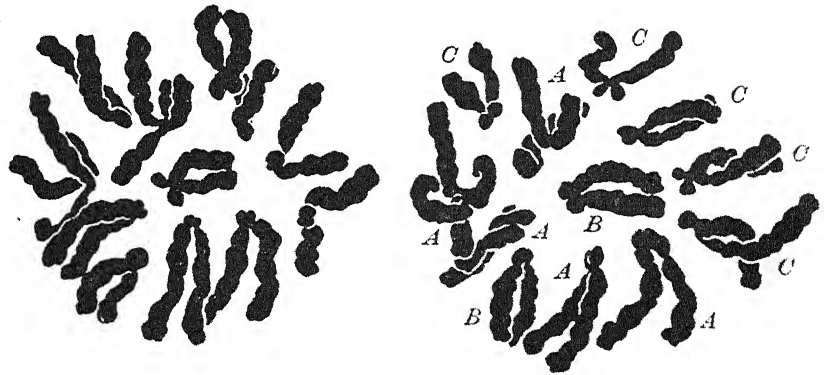


Fig. 8. Two plates from a first division anaphase, drawn separately; chromatids of corresponding chromosomes were still touching. The letters in the right-hand figure show the type of relationship of the metaphase bivalent judged by the scheme illustrated in Fig. 9; five of the *A* type; two of the *B* type; five of the *C* type. They therefore apply to the two corresponding pairs of chromatids in the two figures.

the interpretation of chiasmata at metaphase. Those who doubt the earlier interpretation must see that the chiasma is directly demonstrated where chromatids are seen to be pulling apart, at one end, from mates different from those with which they are associated at the other. This is so in the simplest cases (marked *A* in Fig. 8) where a single chiasma intervenes between the attachment constriction and the distal end of the chromosome (cf. diagram, Fig. 9, *A* 1, *A* 2). The relationships of the

¹ *Fritillaria Meleagris* is peculiarly adapted to study of the anaphase on account of the great lengths of chromatid associated distal to any chiasma; it is therefore always possible to see if there has been a chiasma, or exchange of partners amongst the chromatids between the attachment constriction and the distal end.

separating chromatids give an indication of the more complicated types of relationships with, *e.g.* two chiasmata. Two distinct types should occur: first, those derived from bivalents where the second chiasma restores relations changed by the first (*B* in Figs. 8 and 9) and secondly, those from bivalents where the second chiasma establishes a second new relationship (*C* in Figs. 8 and 9). These types allow of limited possibilities in regard to the equationality and reductionality of the relationships in question¹ (cf. *B* 3 and *C* 3 in Fig. 9), and seem to make the possibility of some of the chiasmata being equational more important (cf. Darlington, 1929 *c*). It will be observed that the long medianly constricted chromosomes (Fig. 8) are both of the type *A* with a single chiasma on either side of the attachment constriction as was actually observed most frequently at metaphase in this type. In Fig. 9 the possibilities with regard to the minor segments have been neglected for simplicity's sake. The anaphase illustrated, however, makes their condition equally clear, and is in accordance with the metaphase interpretation; *i.e.* in some cases, the minor segments of the chromatids associated at the attachment constriction remain closely associated to their ends, while in others the minor segments are sharply turned away from one another. The latter have evidently undergone exchange at a chiasma.

In *Fritillaria* it is particularly clear that the chromatids that are associated at their attachment constrictions at metaphase pass together to the poles and remain in close contact through anaphase until the anaphase of the second division. The first division is therefore essentially a separation of one *pair* of chromatids from another *pair* and the nature of the separation, whether equational or reductional, is determined by the association at the attachment constriction. This general observation seems to be incompatible with Wilson's diagram (1925, p. 520). It is of fundamental importance in considering the relationship of meiosis with ordinary mitosis.

An exact study of the frequency of chiasmata at metaphase is necessarily difficult, but their examination is facilitated by the transparency of the gentian violet stain and by the re-arrangement of the chromatids at each chiasma, each loop lying at right angles to the next one. The interpretation is corroborated by its agreement with the observations of diplotene and diakinesis as well as of anaphase. For example, it may

¹ An *equational chiasma* is one in which the relationships of the chromatids is the same on either side: it cannot therefore correspond to genetical crossing over. A *reductional chiasma* is one which changes the relationships of the chromatids with regard to equationality and reductionality (cf. Darlington, 1929 *c*).

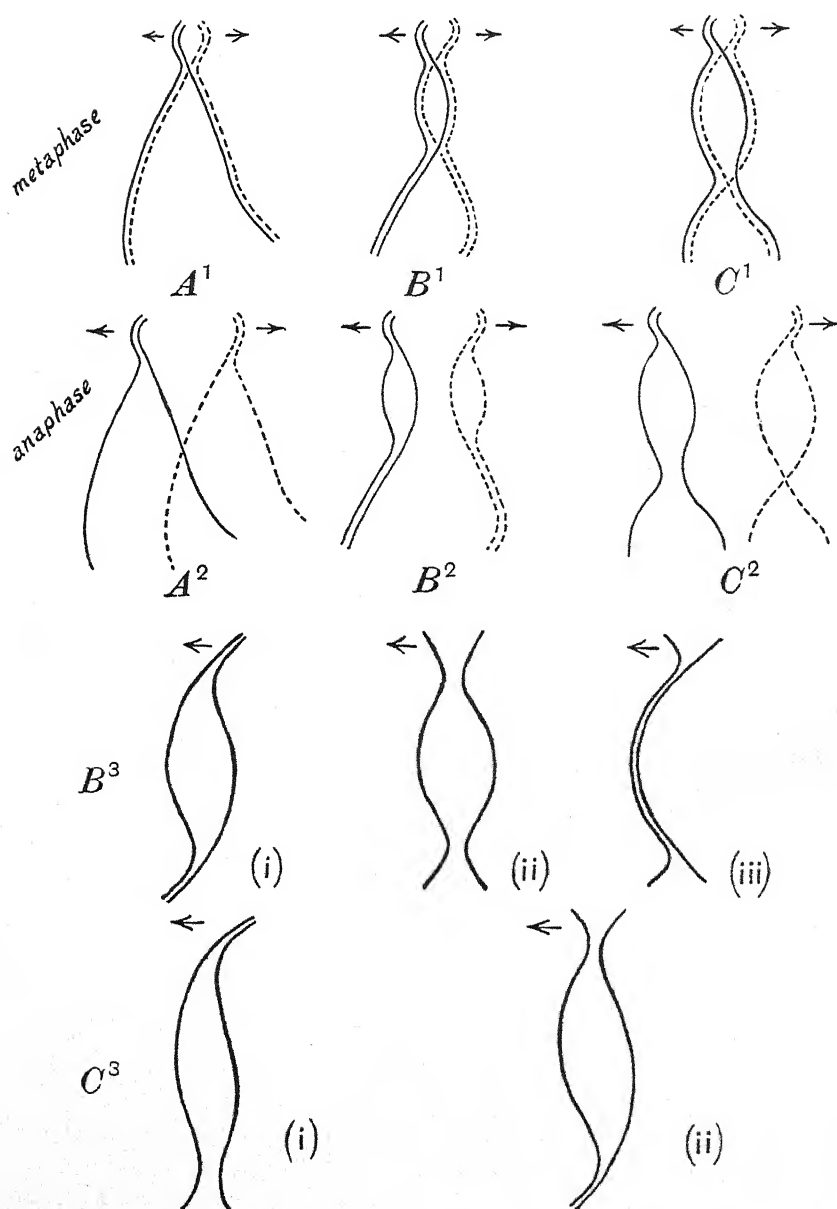


Fig. 9. Diagram illustrating how anaphase separation depends on chiasma relationships at metaphase. Points of attachment marked with arrows. Chromatids associated at the point of attachment pass to the same pole. A^1 and A^2 : bivalent with one chiasma. B^1 and B^2 : bivalent with two chiasmata, the second restoring the relationship that existed before the first. A^1 and B^1 give symmetrical daughter chromosomes. C^1 and C^2 : bivalent with two chiasmata, the second not restoring the chromatid

often appear that the medianly constricted chromosomes are only associated at one point, the attachment constriction; their separation at anaphase reveals the chiasmata by the fact that the two pairs of chromatids associated at the attachment are not the same as the two associated on the other side of the chiasma, *i.e.* distally, for these pairs can regularly be seen pulling apart from one another at anaphase (see diagram, Fig. 9). When, occasionally, only one chiasma is to be found in a medianly constricted chromosome pair, the bivalent is characteristically orientated in relation to the spindle (Fig. 7 *e*), for the loop or chromosome segment including the attachments is always placed so that the corresponding attachments lie in the axis of the spindle (instead of all four arms emerging from the chiasmata, as normally, in the plane of the equatorial plate); and these two arms pass to the pole at anaphase, no doubt, without any separation of their constituent chromatids (cf. Aneuploid *Hyacinthus*, Darlington, 1929 *c*). It will be seen therefore that, questions of orientation being of the utmost importance in determining a chiasma, interpretation for statistical purposes must be confined to side views of metaphase. The following table is the result of an attempt to estimate chiasma-frequency statistically on a small scale.

The method of interpretation is admittedly difficult and liable to error, but it is surely better to describe metaphase, as Belling (1927) and we ourselves have attempted to, in terms of observed relations of the structures taking part, than to conceal these relations—and the whole importance of the study—under an arbitrary terminology. The terms “anaschistic” and “diaschistic,” for example, do not seem to correspond either to types of behaviour before metaphase or to types of structure at this stage. They correspond to accidental similarities in the subsequent behaviour of the chromosomes without regard to its significance in development.

Statistical studies of chiasmata have already been made by Belling (1927) in *Hyacinthus*, where interpretation can reach a high level of

relationship that existed before the first: daughter chromosomes do not correspond. B^3 and C^3 : the five arrangements of identical chromatids possible with two chiasmata at metaphase (types *B* and *C*); the sister pair of chromatids is not shown. B^3 : (i) type with both chiasmata reductional: first division reductional; (ii) type with both chiasmata equational: first division equational; (iii) type with both chiasmata reductional: first division equational. C^3 : (i) type with one equational chiasma, the other reductional: first division reductional; (ii) type with one equational chiasma, the other reductional: first division equational. Other geometrical types not given are biologically equivalent to these five. The whole and broken lines in A_1 to C_2 distinguish the chromatids according to their polar destination and have no relation to their equationality and reductionality.

TABLE I.

Table of chiasma¹ frequencies in *Fritillaria Meleagris*.

Chromosome type ²	Minor limb		Major limb				
	0 Xta	1 X	0 Xta	1 X	2 Xta	3 Xta	4 Xta
(1) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	6	3	1	—
(2) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	5	5	—	—
(3) 2 m.	—	2	—	1	1	—	—
10 s.	ca. 3	ca. 7	—	6	4	—	—
(4) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	5	4	1	—
(5) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	7	3	—	—
(6) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	5	5	—	—
(7) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	4	3	3	—
(8) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	3	4	2	1
(9) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	4	5	1	—
(10) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	6	3	1	—
(11) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	7	3	—	—
(12) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	7	3	—	—
(13) 2 m.	—	2	—	2	—	—	—
10 s.	ca. 3	ca. 7	—	5	5	—	—
(14) ³ 2 m.	—	2	—	2	—	—	—
10 s.	5	5	—	3	4	3	—
(15) ⁴ 2 m.	—	2	—	2	—	—	—
10 s.	2	8	—	6	3	1	—
Totals 30 m.	0	30	0	29	1	0	0
150 s.	ca. 46	ca. 104	0	79	57	13	1

certainty. At present, however, our object is not to correlate their frequency with crossing-over (in this or any other organism) but to find some indication of the influences concerned in the concentration of the chiasmata in the neighbourhood of the attachment.

These results, in one way, support the assumption that chiasmata are originally formed with a frequency diminishing with the distance from the attachment constriction. Thus the total number of chiasmata found in 150 major segments examined was 236, in the same number of minor segments approximately 104. Yet the minor segments are less than an eighth of the length of the major.

¹ Signified by X in the table.

² m. are the two pairs of chromosomes with median constrictions. s. are chromosomes with subterminal constrictions.

³ Illustrated in Fig. 5.

⁴ Illustrated in Fig. 6.

The alternative view suggested earlier (Darlington, 1929 *b*) is that chiasmata have moved in this case towards the attachment, just as they have so often been shown to move away from it in "terminalisation" (Wenrich, 1916, and Darlington, 1929 *c*). This random formation is suggested by the fact that the chiasma frequency in each of the arms of the medianly constricted chromosomes is 1.02, while in the long arms of the subterminally constricted chromosomes, which are about one and a half times as long, the chiasma frequency is 1.57. The other frequencies found are in accordance with this view if we assume cancellation of chiasmata in the major segments but not in the minor. But the occasional occurrence of distal chiasmata at metaphase is difficult to reconcile with the assumption of chiasma-movement for they are not related even to the secondary constrictions.

We are therefore left with the conclusion that loops are formed more frequently, or successive loops arise in different planes more regularly in the neighbourhood of the attachment constriction. The extreme example of this concentration is shown in the case of a sub-terminally constricted chromosome bivalent (Fig. 7 *g*) which is associated with a chiasma in the minor segment only, the major segments lying free. Such variations reveal *Fritillaria* in an intermediate position between *Mecostethus* (*Stethophyma*) and the rest of observed material¹.

How then is one to explain chiasma formation in *Fritillaria* in terms of any other known phenomenon? A failure of synchronisation of looping in different parts of the chromosome is the most obvious suggestion, but the question must remain for the moment unanswered. A comparative study of chiasma formation in related forms will perhaps throw light on this problem and on wider problems of chromosome behaviour and genetical crossing-over.

Four possible corollaries of the localisation of chiasmata are worth mentioning.

(*a*) The possibility of a concentration of crossing-over in one region of a chromosome just as chiasma formation is concentrated in one region in *Fritillaria Meleagris* is worth considering. The genetical evidence in the "complex heterozygote" *Oenothera* species (Renner, 1925, 1927) points to the existence of one restricted part of each chromosome in which crossing-over takes place freely, and a "rest" in which crossing-over takes place very rarely. If this is the case, as the result of a

¹ As far as one can say in the absence of any study of *Mecostethus* undertaken from the same point of view (cf. e.g. Janssens, 1924).

concentration of crossing-over towards the ends of the chromosomes in *Oenothera*, then the condition of the complex heterozygotes can be more clearly visualised. Each pair of segments of chromosome (cf. Darlington, 1929 *a*) in a type which has long been self-pollinated will consist of a generally homozygous terminal region and (in a structural hybrid) a heterozygous region, in the neighbourhood of the attachment constriction (which is approximately median in *Oenothera*). Rare mutants (such as *biennis-sulfurea*) will then arise, as Renner believes (1927), solely as a result of crossing-over which will take place in an intermediate zone. Segmental interchange itself, and hence half-mutants, are not improbably caused by single crossing-over within homologous interstitial segments, one of which has been translocated to an interstitial region of a non-homologous chromosome. These questions will be considered in detail at a later date.

(b) Where chiasmata are originally formed at random, as in *Secale*, *Hyacinthus* and probably *Tradescantia*, fragmented chromosomes, or, in triploids, the smaller chromosomes, pair less regularly at meiosis than whole large chromosomes because, as has been suggested (Darlington, 1929 *b* and *c*), the association of chromosomes depends on the establishment of a chiasma between their chromatids. The same conclusion may be derived on genetical grounds from observations of the segregation of fragmented Y-chromosomes in *Drosophila* (Stern, 1929). Where, however, the chiasmata are localised, such a modification in pairing by fragmentation, or differentiation in the behaviour of a smaller chromosome in a triploid form, is not to be looked for. If the new attachment constrictions of fragments continue to induce a localisation of chiasmata then the fragments will pair as regularly as the whole chromosomes. This will make fragmentation more important as a means of evolutionary change.

(c) Localisation of the chiasmata near the attachment constriction is the condition opposed to terminalisation of chiasmata, for, while terminalisation gives the minimum of separation of chromatids at anaphase, localisation, as in *Fritillaria*, requires the maximum length of separation—the most complex movement—at this stage. The rarity of the *Fritillaria* type, taken together with the commonness of terminalisation, which ensures the greatest ease of separation, suggests that the terminalised chiasma is an adaptation to the conditions of meiosis promoting regularity (for which it is indispensable in ring-forming species such as *Oenothera* and *Rhoeo*). Localisation, on the other hand, can exist in *Fritillaria*, for fertility in sexual reproduction is not necessary for the maintenance of the species.

(d) In *Fritillaria Meleagris* all the chromosomes are uniform in regard to their abnormal properties of chiasma distribution. This must therefore be a general genetic property of the plant, analogous perhaps to the single factor which, according to Gowen, may inhibit crossing-over in *Drosophila*.

SUMMARY.

1. *Fritillaria Meleagris* has twelve pairs of chromosomes, two with median, ten with more or less sub-terminal attachment constrictions. One pair has a second constriction.

2. At diplotene in the prophase of meiosis chiasmata are not formed at random but with a greater frequency in the neighbourhood of the attachment constriction. The two chromosome types are therefore distinguishable at this stage by the distribution of their chiasmata.

3. There is no definite change in the relationships of the chromatids (*i.e.* no movement of chiasmata) between diplotene and metaphase.

4. The great contraction of the chromosomes conceals the details of their relationships, at least in polar views of metaphase, but it has been possible to make a small statistical study of the frequency of chiasmata from side views (see Table I). This, and the separation at anaphase, corroborate the chiasma interpretation of the structure of the bivalent chromosomes at metaphase.

5. It follows that chromosome behaviour at meiosis must be examined in relation to the possibility of differential frequency of chiasmata as well as of their movement after formation. A knowledge of both these circumstances is necessary before one can consider the analogy with crossing-over results in a particular species.

6. In the related species *Fritillaria imperialis* chiasmata are formed at random. An account of behaviour in this species will appear later.

7. The bearing of this work on the *Oenothera* problem is indicated.

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EXPERIMENTS ON THE GENETICS OF WILD POPULATIONS.

I. *PLANTAGO MARITIMA*.

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(With Two Plates and One Text-figure.)

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INTRODUCTION.

THE present paper may be regarded as introductory to a study of the effects of environmental conditions on isolated populations of *Plantago maritima* L. in the British Isles. The particular aspects of the problem to be considered here are the general distribution of the species in Britain and the growth-forms which inhabit that area.

Any form of isolation which separates parts of a species-population from the mass undoubtedly aids in the formation of habitat types; further, the effect of environmental factors in bringing about local differentiation must also play an important part. It seems reasonable to assume that if a cross-fertilised species possessed a continuous distribution extending through a uniform environment, undivided by mechanical or other barriers to free sexual reproduction, the chances are small that such a population would be split into genotypically distinct groups because of the tendency to a general diffusion of the existing characters throughout the entire population. In nature, however, such a combination of conditions is rarely encountered, and local differentiation is usually attributed to some form of isolation or to the influence of the environment. Nevertheless, direct experimental evidence concerning the inherited differences between portions of a natural species-population is extremely limited.

Conclusions which have been based on experimentation conducted for a very limited period (a few generations) are sometimes utilised to elucidate problems connected with the inter-relationship of the plant and its environment. The experimental conditions, however, do not truly represent the natural conditions, for, in the wild, the interactions of plant and environment operate over a long period of time.

The research to which this paper forms an introduction represents an attempt to overcome the time-factor difficulty by means of a study, in culture, of parts of a species-population from different wild habitats. Each part will presumably have been subjected to the influence of a particular environment for many generations, and, by the examination of collections from isolated but apparently similar habitats, it should be possible to estimate the importance of isolation as a factor in the differentiation of local types.

MATERIAL.

For the purposes of the present investigation the species *P. maritima* has been utilised, since it possesses certain qualities necessary for a research of this nature:

- (1) A continuous coastal and localised inland distribution in the British Isles.
- (2) Cross-fertilisation as the normal means of sexual reproduction; in many cases obligatory, owing to a low degree of self-fertility.
- (3) Characters which can be measured with some degree of accuracy.

DISTRIBUTION OF *P. MARITIMA*.

The species occurs in Europe, Central Asia, America and in South Africa. The British population of *P. maritima* has been separated from the main mass of the species on the Continent for a very considerable time, and has, since its isolation, undergone further division into localised populations. Its distribution is continuous throughout the maritime regions, but inland it is decidedly scattered, being chiefly confined to high altitudes. Nevertheless it is incorrect to conclude that inland the species is restricted to the higher mountains, since the present writer has collected it from central Scotland at elevations of over 2500 feet and also as low as 400 feet.

If reference is made to the floral history of Britain, the discontinuous inland distribution of *P. maritima* can, at least partly, be explained. It is still a matter of considerable doubt whether, during the Pleistocene period, the flora of the British Isles was exterminated, or whether some

portion survived in the south of England during the period of maximum glaciation. It is, however, certain that the greater part of Scotland was denuded at that time of its previous flora, and the development of its present vegetation commenced only when the Ice Age was succeeded by more congenial climatic conditions. Matthews (1923, 1924) has demonstrated the lines of plant invasion into Britain from the European Continent, and he concludes that overland migration is mainly responsible for our present flora, although he believes that part of the flora survived the period of maximum glaciation, at least in the South of England. Chevalier (1923) suggests that the northward movement of species after the retreat of the ice had not been completed when the Channel was formed. Thus the further spread of Continental plants into England, except for occasional subsequent introductions, would be prevented by this natural barrier. Turrill (1927) holds that the flora of the islands of St. Kilda "as represented by the dominant heath moor types, survived the Ice Age in the islands, either these escaping glaciation owing to their oceanic position or plants continuing to exist on local nunataks." Turrill (1928): "That the same is true for the heath-moor flora, and one might add the aquatic and marsh flora, of Foula seems even more likely." Woodhead (1929) points out that, even at the glacial climax, there were parts of the Pennines free from ice. In his paper on the Pennine peats (1924) he suggests that "as the Southern Pennines was an unglaciated area during the Ice Age these moorland species would for the most part persist through that period," but, as he writes (1929), "we have no records preserved to us of the vegetation of the area at this early period." On the other hand Reid (1899) infers that, during the glacial climax, the flora of Britain was exterminated except in the south. He writes: "The result seems to have been the total blotting out of the flora over the area north of the Thames and Severn, with the possible exception of certain high hills which rose above the ice. Even these were probably so smothered with snow that only the steeper crags were bare in summer."

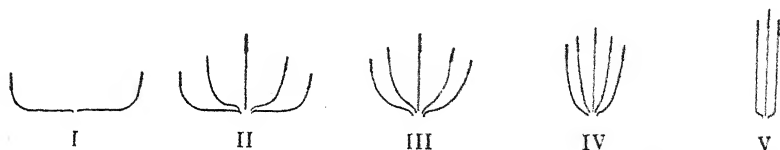
The difficulty of estimating the age of the flora of Britain is obvious. It is, however, the conditions prevailing during the period of maximum glaciation which are of importance in this connection, and whether the species *P. maritima* survived the extreme conditions of that period, as a nunatak species, is very doubtful.

The present inland distribution of *P. maritima* is, therefore, apparently a remnant of a former more general distribution. It is probable that this species followed the retreating ice at the close of the glacial

period, and, owing to its xerophytic nature, it would be well qualified to withstand the prevailing conditions of physiological drought; this supposition is borne out by the fact that the species is frequently associated inland, at high altitudes, with other plants of the sea shore. Presumably the British *P. maritima* originated from a continental stock which spread, by overland migration, over the greater part of the country in early post-Pleistocene times, subsequent environmental changes having been responsible for the present restricted inland distribution. There appears to be little doubt that competition with other species has exterminated *P. maritima* from the intermediate stations, since it can grow vigorously at low elevations under mesophytic conditions both in culture and in the wild, provided that competition is not excessive; this illustrates, as Salisbury (1929) remarks, the fact that plants grow not where they would but rather where they must.

GROWTH-FORMS.

The species *P. maritima* comprises many growth-forms which range from the decumbent to the erect. For purposes of identification they have been classified into five groups (Text-fig. 1), although actually there is no sharp line of demarcation between one form and the next in the series.



Text-fig. 1. Five types of habit of growth represented diagrammatically.

Form I (Plate I, No. 1). *Spikes*: restricted to periphery. *Scapes*: all markedly decumbent.

Form II (Plate I, No. 2). *Spikes*: general. *Scapes*: peripheral markedly decumbent, becoming erect towards centre of plant.

Form III (Plate I, No. 3). Similar to Form II but peripheral scapes less decumbent.

Form IV (Plate I, No. 4). *Spikes*: general. *Scapes*: peripheral sharply ascending.

Form V (Plate II, No. 5). *Spikes*: general. *Scapes*: erect.

The different combinations of characters, e.g. leaf length, leaf breadth, etc., exhibited by plants of the same growth-habit make the total number of forms in the species very extensive. Under natural conditions the

plants forming a habitat population may sometimes be so modified by extreme environmental conditions as to assume a phenotypic similarity which masks the presence of the various forms, and it is only by bringing such plants into the more congenial environment of the experimental garden that the differences in type between the members of the population become evident.

COLLECTION AND EXAMINATION OF MATERIAL.

Observations made on populations grown under controlled conditions have indicated that a culture of a population raised from seed collected in the wild, probably gives a better conception of the genotypical composition of that population than does a culture of plants collected in the mature state. In the latter case the phenotypical uniformity of the plants forming a population is sometimes exaggerated, owing to the suppression of certain phenotypes by the environmental conditions of the wild habitat. Moreover, for the comparison under a uniform environment of populations from distinct habitats, the cultures raised from seed have the advantage of developing under the experimental conditions from the time of sowing. In the case of collected plants, on the other hand, the uniform environment acts only from the time of transplantation and, as a result, the time required to nullify the modificatory influence of the previous environment is indefinite.

Under cultivation all the populations received similar treatment. The samples were sown in a greenhouse, and the seedlings were transplanted at an early stage of growth into boxes, whence they were transferred to the experimental garden; they were spaced 1.5 feet (46 cm.) apart in rows at 2 feet (61 cm.) intervals. As a result of this method the seedling death-rate was practically negligible. The populations were examined during both the first and second year of their growth.

When comparing the measurable characters of the populations the mean was taken as the average value for the character in question, since it gives weight to the extreme variations, and for this reason a greater significance was attached to this figure than to the mode. In comparing the means the differences were taken to be significant only when they exceeded five times the Standard Error of the difference. It may be concluded, therefore, that populations with means which differ by this amount do not form a homogeneous group, the variability being too high to result from the operation of chance alone.

MATERIAL IN NATURE.

The present paper deals only with plants derived from a single locality on the east coast of Scotland. This area, though very limited in extent, was ecologically divided into two regions:

(1) Exposed rock immediately above the normal high-tide mark. The plants of *P. maritima* were sparsely distributed, and only occurred where small quantities of organic matter had accumulated. They resembled each other closely and were characterised by a dwarf, more or less decumbent, habit of growth.

(2) An adjoining grassy slope. *P. maritima* was here growing in a dense association, mainly composed of varieties of *Festuca ovina* L., which attained an average height of 9 inches. The majority of the plants of *P. maritima* exhibited the upright growth habit, but some type differentiation was in evidence.

MATERIAL IN CULTURE.

As previously mentioned the plants growing on the exposed rock were somewhat scattered, and this fact, in conjunction with the extreme environmental conditions, reflected adversely on seed production, with the result that the number of plants available for the culture experiments was limited to 50 from each habitat. Notwithstanding this scarcity of material in culture, sufficient evidence has been forthcoming to indicate that the plant populations from the two areas neither formed a homogeneous mass, nor differed so greatly as their parent populations. The plants were examined and measurements of certain characters made at intervals during a period of two years. The plants of each population were classified at the end of their second year's growth into the types previously described in this paper. The results expressed as percentages are given in Table I.

TABLE I.

Types within the populations.

Type	% of types in population	
	<i>P</i> 11 (grass)	<i>P</i> 12 (rock)
1	15	61
2	40	18
3	30	19
4	15	2
5	0	0

Table I shows the difference as regards habit of growth between the two populations. Although the typical Class 5 habit of growth was not

represented in either population, one plant of *P* 11 closely approached the required standard.

In Table II the measurements of the various characters are given; the leaf data are confined to the first part of this table and are followed by the scape and spike measurements. The headings leaf breadth and leaf length are self explanatory, but leaf height and leaf spread require some further qualification. The leaf height of a plant was taken to be the vertical distance from the ground level to the upper leaf limit, and the

TABLE II.

Leaf and scape measurements for populations P 11 and P 12.

Character	Popula- tion no.	Date of measurement	Range		Mean	Difference between means	Coefficient of variability
			Min.	Max.			
			Leaf measurements				
Breadth in mm.*	<i>P</i> 11	31. v. 28	7	17	10.3±0.40	2.4 ±0.53	25.4 ±2.94
	<i>P</i> 12	"	4	14	7.9±0.35		32.8 ±3.41
	<i>P</i> 11	21. vi. 28	6	16	10.4±0.36	2.3 ±0.48	21.2 ±2.37
	<i>P</i> 12	"	4	15	8.1±0.34		30.9 ±3.20
Length in cm.†	<i>P</i> 11	31. v. 28	15	36	23.6±0.70	1.8 ±0.90	19.2 ±2.15
	<i>P</i> 12	"	12	37	21.8±0.59		19.6 ±1.90
	<i>P</i> 11	21. vi. 28	20	37	26.3±0.68	0.6 ±1.01	16.3 ±1.86
	<i>P</i> 12	"	16	40	25.7±0.75		21.4 ±2.14
Height in inches	<i>P</i> 11	1. ix. 27	3	10	6.4±0.34	2.0 ±0.47	34.6 ±4.13
	<i>P</i> 12	"	2	7	4.4±0.34		51.8 ±6.70
	<i>P</i> 11	15. v. 28	2	8	4.2±0.23	1.6‡±0.28	35.2 ±4.14
	<i>P</i> 12	"	1	8	2.6±0.18		50.8 ±5.90
Spread in inches	<i>P</i> 11	1. ix. 27	10	28	18.1±0.62	0.5 ±0.79	22.1 ±2.46
	<i>P</i> 12	"	10	23	17.6±0.50		19.4 ±2.06
	<i>P</i> 11	15. v. 28	9	21	15.9±0.43	1.4 ±0.60	17.7 ±1.99
	<i>P</i> 12	"	9	22	14.5±0.42		21.9 ±2.15
Ratio spread/height	<i>P</i> 11	1. ix. 27	1.7	4.3	3.2±0.13	1.4‡±0.27	26.6 ±3.03
	<i>P</i> 12	"	1.7	8.0	4.6±0.23		35.0 ±3.99
	<i>P</i> 11	15. v. 28	2.3	9.0	4.2±0.18	2.6‡±0.38	28.3 ±3.30
	<i>P</i> 12	"	3.0	14.0	6.8±0.34		37.1 ±4.03
Scape measurements							
Length in inches	<i>P</i> 11	5. vii. 28	12	17	14.4±0.22	1.8 ±0.37	9.4 ±1.06
	<i>P</i> 12	"	8	19	12.6±0.31		17.9 ±1.77
	<i>P</i> 11	1. viii. 28	13	19	15.2±0.23	1.8 ±0.42	9.6 ±1.08
	<i>P</i> 12	"	8	19	13.4±0.36		19.7 ±1.94
Height in inches	<i>P</i> 11	1. ix. 27	7	18	11.5±0.50	2.6 ±0.73	28.8 ±3.30
	<i>P</i> 12	"	4	18	8.9±0.54		39.4 ±4.83
	<i>P</i> 11	5. vii. 28	8	21	13.7±0.44	3.6‡±0.64	20.2 ±2.36
	<i>P</i> 12	"	5	19	10.1±0.47		34.2 ±3.65
Spread in inches	<i>P</i> 11	1. viii. 28	8	21	15.9±0.44	4.4‡±0.72	17.7 ±2.04
	<i>P</i> 12	"	5	22	11.5±0.57		36.6 ±3.94
	<i>P</i> 11	1. ix. 27	8	23	14.8±0.50	1.4 ±0.73	21.6 ±2.47
	<i>P</i> 12	"	6	25	16.2±0.52		21.6 ±2.40
Ratio length/height	<i>P</i> 11	5. vii. 28	0.8	1.9	1.1±0.04	0.3‡±0.06	21.8 ±2.54
	<i>P</i> 12	"	0.8	2.2	1.4±0.05		24.1 ±2.42
	<i>P</i> 11	1. viii. 28	0.8	1.7	1.0±0.03	0.3‡±0.05	18.0 ±2.08
	<i>P</i> 12	"	0.8	2.2	1.3±0.05		26.2 ±2.67
Ratio spread/height	<i>P</i> 11	1. ix. 27	0.8	3.5	1.1±0.09	1.0‡±0.15	48.3 ±6.44
	<i>P</i> 12	"	0.7	5.1	2.1±0.13		41.4 ±5.10

* The broadest leaf on each plant. † The longest leaf on each plant. ‡ Difference significant.

leaf spread was the greatest distance, across the plant, between the apices of opposite leaves. The scape measurements are those of the combined peduncle and rachis. The scape height is the vertical distance from the ground surface to the apex of the tallest spike, and the scape spread is the maximum distance through the centre of the plant between the apices of opposite marginal spikes.

From a comparison of the means of leaf height and scape height of the two populations, it is evident that there is a decided tendency towards a lower growth habit in the case of the rock population.

Although most importance is attached to the mean values, it would be inadvisable to disregard the figures given under the "range" column since it is these figures which indicate the types possessed by the populations. We see from the "difference between means" column that there is a significant difference between the leaf heights and the scape heights respectively for the two populations in their second year of growth. Nevertheless, from the "range" column of the same dates it is evident that the differences are not due to the absence of tall plants in the rock population, but to the preponderance of the tall type and the lack of low growth forms in the grass population. The difference in growth habit is perhaps more pronounced when the ratios leaf spread/leaf height, scape length/scape height, and scape spread/scape height respectively, are compared for the two populations; these ratio differences are significant according to the chosen standard. In culture, the mean leaf breadth for *P* 11 was greater than that for *P* 12, but the difference is not significant; the difference in leaf length is obviously not significant. Under the wild conditions, however, one of the most marked distinctions between the two populations was the leaf form. The leaves of the rock population were short and almost cylindrical (Plate II, fig. 6 *a*), while those of the grass population were long and dorsiventral, and exhibited obvious signs of etiolation. In transverse section the two leaf types were distinct. Plants which had been taken from the rock habitat and cultivated for a year lost their characteristic cylindrical leaves and became indistinguishable from plants collected from the grass habitat (Plate II, fig. 6 *b*). All the plants of populations *P* 11 and *P* 12, raised at Corstorphine, exhibited the dorsiventral leaf type, but variation in leaf succulence was observed. It was demonstrated that the fleshy habit of the leaves could be induced experimentally by watering the plants with a 3.5 per cent. solution of sodium chloride.

In addition to the examination of measurable characters, observations were made on the floral characters, the time of flowering, the leaf shape,

and the presence or absence of hairs on both the leaves and scapes, but, although considerable variation existed, no definite distinction between the two populations was found.

DISCUSSION AND CONCLUSIONS.

It is unlikely that a part of a species-population will represent the genotypic composition of the whole, and, therefore, when a part is prevented from crossing with the bulk of the species it may be expected that the separated portion will differ in some way from the species as a whole. The number of agencies, however, involved in the differentiation of species and populations within species must be large, and the assumption is unwarranted that any one of these can be, to the exclusion of all others, the sole controlling factor. The influence of environment is doubtless of considerable importance, but the interactions between the environment and the organism are not really fully understood. Do environmental conditions, acting on a population for many years, gradually change the genotypic composition of that population, or are individual plants occasionally altered genetically as a result of the direct influence of the environment? Is the action of the environment merely selective? From the work of Turesson and others, and from the writer's own observations on *P. maritima* and species of Gramineae, it is apparent that the local differentiation within a species-population results from the suppression of certain types which are unable to survive in the particular habitat. It is improbable, however, that the eliminating influence of the environment could be wholly responsible for the definiteness of type so often exhibited by local populations (*e.g.* the British freshwater fishes), since it is difficult to imagine what survival value certain characters possess. One is therefore led to assume that, in some cases at least, the partial or even complete isolation which may follow the selective processes of the environment is of considerable importance in establishing the apparently non-essential characters of local populations.

The two populations at present under discussion afford an example of population differentiation unaccompanied by spatial isolation. In nature they occupied two sharply defined, adjoining areas which supported populations of *P. maritima* of different growth habit. The environment had modified both populations considerably, but the effect was more accentuated in the case of the exposed rock plants, the types of which could only be distinguished after a period of transplantation. The progeny of the rock population possessed a larger proportion of low-growing forms than that of the grass population, but the latter contained no

classified type which was not also present in *P* 12 (Table I). The fact that there was a tendency in culture towards a lower habit of growth on the part of the rock population is of significance when it is remembered that the modificatory influences of the wild environment produced a population of dwarfs. There was a decided difference, however, between the cultivated material and that in the wild, since the former exhibited types ranging from the decumbent to the almost erect, whereas in the latter a more or less uniform dwarf population occurred. As Turesson (1922) points out: "the morphological parallelism between the modifications and the hereditary variations offers an additional proof of the control of the environmental factors upon the direction of the differentiation process of the habitat types." Again with reference to Table I, it is apparent that the low growth habit is favoured in the exposed situations and the more upright type in the grass habitat.

Although it is "the sum total of genes which doubtless determine the presence or absence of a certain form in a certain habitat" (Turesson (1922)) it has been suggested (Gregor and Sansome (1927)) that the survival of low-growing forms of *Lolium perenne* L., under conditions of severe grazing, was apparently due to the possession by such plants of a phenotype capable of survival under these conditions. In the case of *P. maritima* it is also possible that occasionally the phenotypical characteristics of growth-forms determine their survival, and one is therefore led to attach some importance to the value of any genotype as represented by a particular phenotype.

SUMMARY.

1. The British distribution of *P. maritima* is continuous throughout the coastal regions, but is localised inland.
2. The species is an aggregate of many growth-forms representing various combinations of characters. Growth habit ranges from the decumbent to the erect form, and five types have been described and figured.
3. Plants derived from a single area on the east coast of Scotland were studied. This locality, though very limited in extent, was ecologically divided into two regions: (a) an exposed rock habitat; and (b) an adjoining grassy slope.
4. Although the two habitats were not spatially isolated, population differentiation had occurred.
5. In the wild, the environment had modified both populations considerably, but the effect was more marked in the case of the rock population.

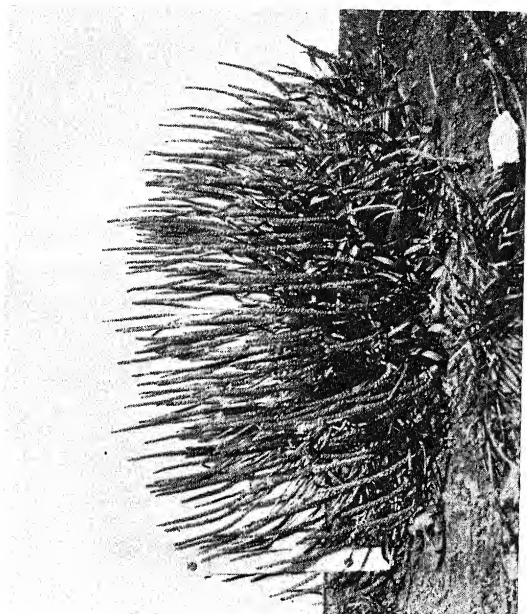


Fig. 2.

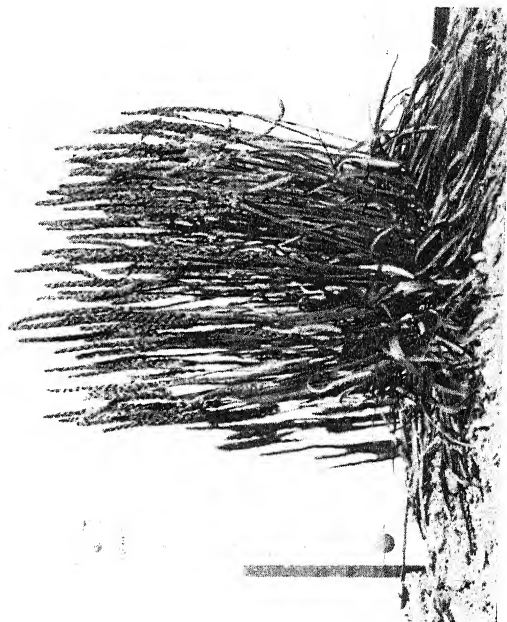


Fig. 4.

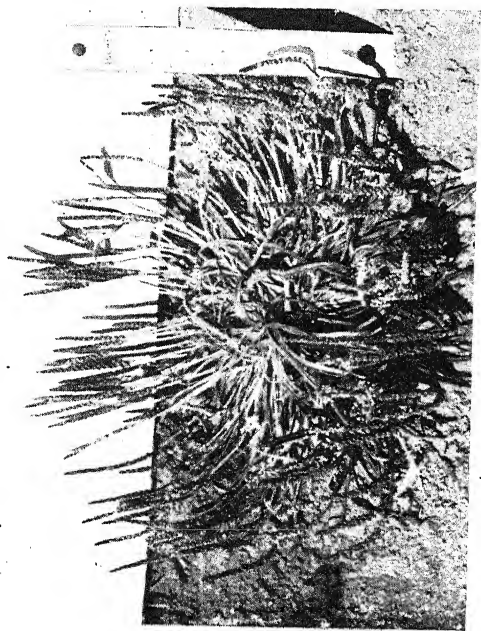


Fig. 1.

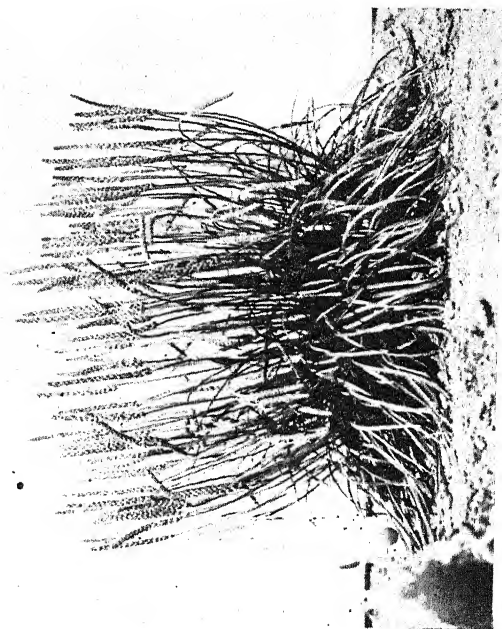


Fig. 3.



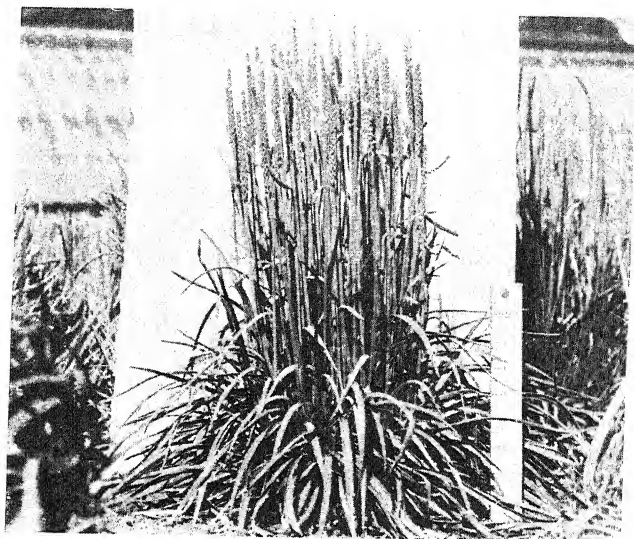


Fig. 5.

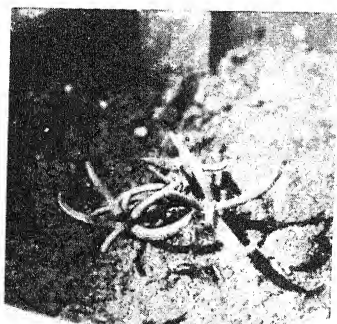


Fig. 6 (a).

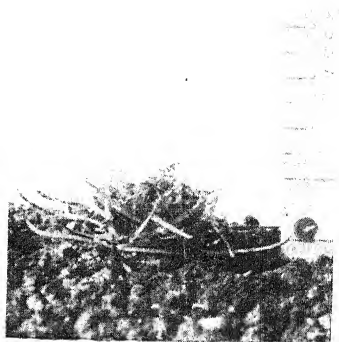
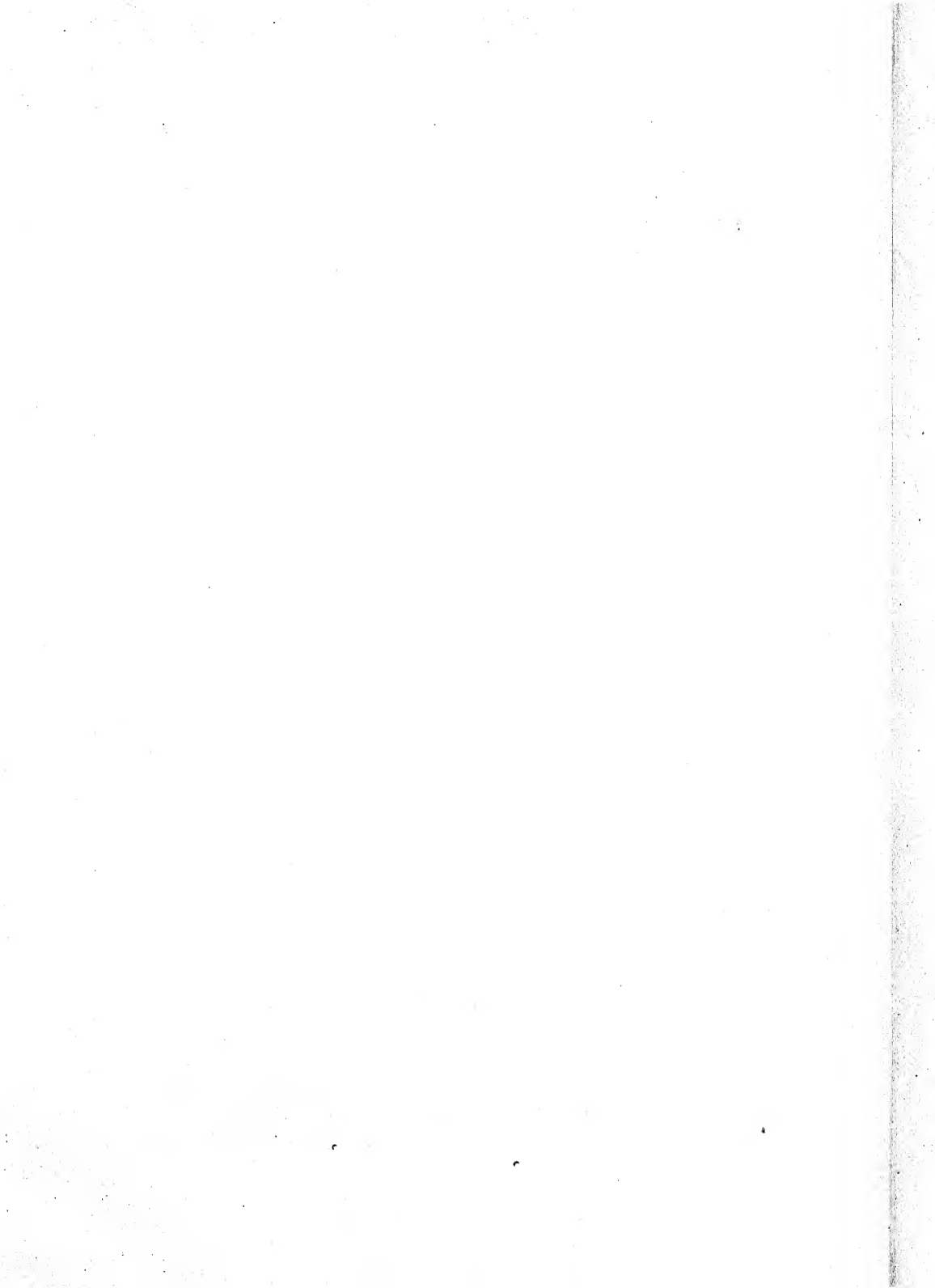


Fig. 6 (b).



6. In culture the rock populations possessed a larger proportion of low-growing forms than did the grass population, but the latter contained no classified type which was not also present in the former.

7. A phenotypic parallelism between the modificatory effect of the environment on the populations in the wild and the growth-forms present in the cultured populations was observed.

8. It is possible that occasionally the phenotypical characteristics of growth-forms determine their survival, and some importance should be attached to the value of the several genotypes represented by a particular phenotype.

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EXPLANATION OF PLATES I, II

Plantago maritima.

- Fig. 1. Form I.
 Fig. 2. Form II.
 Fig. 3. Form III.
 Fig. 4. Form IV.
 Fig. 5. Form V.

Fig. 6 a. Plant *E* 6 collected from the exposed rock habitat. Photo: 17. viii. 26.

Fig. 6 b. The same plant in culture. Photo: 3. iv. 28.

NOTES ON THE PROGENIES OF VARIOUS POTATO HYBRIDS.

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(With One Text-figure.)

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INTRODUCTION.

In experiments at the Plant-Breeding Station, Corstorphine, to obtain new and improved varieties of potato, plants of many varieties have been hybridised. In the course of this work data have been collected with reference to the modes of inheritance of several characters in various progenies, and an endeavour has been made to discover which varieties, when hybridised, give the best results as judged by economic standards.

To obtain satisfactory comparisons of hybrid progenies it is necessary to have some assurance that the seedlings are all hybrid plants of known parentage. Therefore in all cases where the variety used as female parent produces viable pollen, the flowers were emasculated before opening and thereafter protected by pergamine bags. When they became fully open and receptive, pollen was taken from the anthers of the male parent and applied to the stigma of the female. The pergamine bag was then replaced and allowed to remain in position until the berries had set. Where the female parent was found to be devoid of viable pollen emasculation was omitted, but the flowers were protected as before. Every precaution was taken to prevent the introduction of foreign pollen, and the progenies which will be enumerated can be regarded as true hybrids of the parentage stated.

That all modern commercial varieties of the potato are more or less

genetically impure has been suggested by Robb(s) and others, and this view appears to be generally accepted. In the following notes attention will be confined to a few outstanding characters.

A list of the parent varieties of hybrids described in this paper, together with a brief description of each parent, with regard to five characters, viz. flower colour, tuber colour, flesh colour, tuber shape and eye depth is presented in Table I. The first eight varieties in the list are well-known commercial varieties, and further information concerning them can be obtained from *Miscellaneous Publications*, No. 3, of the Board of Agriculture for Scotland(2), from *Potato Varieties* by R. N. Salaman(9), or from *The Potato* by T. P. McIntosh(7).

TABLE I.

List of parent varieties.

Variety	Flower colour	Tuber skin colour	Tuber flesh colour	Tuber shape	Eye depth
British Queen	White	White	White	Oval	Shallow +
Epicure	White	White reddish purple	White	Round	Deep
Flourball	White, tinted purple	Pale reddish purple	White	Round	Medium
Glassel Beauty	White	Pale reddish purple	White	Round	Shallow
Golden Wonder	Purple, tipped white	Russet (white)	White +	Kidney	Shallow
Great Scot	White	White	White +	Round	Medium -
Kerr's Pink	White	Pink	White	Round	Medium +
Up-to-date	Light reddish purple	White	White	Oval	Shallow
Bell	White	White	Pale yellow	Round	Medium
39 (15)	Reddish purple, tipped white	White	Pale yellow	Kidney	Shallow
800 (2)	White	White	White	Round	Medium +
993 (a) (4)	White	White	Pale yellow	Oval -	Shallow +

White + =white inclining to be pale yellow

Oval - =oval inclining to be round

Shallow + =shallow inclining to be medium

Medium - =medium inclining to be shallow

Medium + =medium inclining to be deep

FLOWER COLOUR.

Salaman(9) has distinguished two types of flowers which are commonly known as white, viz. "pure white" and "tinted white." The latter possesses a small amount of pigment, more readily found in the bud, and is usually confined to the under surface of the petal. It is claimed by the above author that the two types referred to are distinct, and that their union by hybridisation would result in the production of a certain number of offspring bearing heliotrope-coloured flowers.

Purple in the flowers, according to East (5), is dominant to its absence, and Salaman (10) suggests that heliotrope colour is due to two factors and purple to a third.

Fertility in the potato is controlled by genetic factors (Salaman (11)) which include a dominant factor inhibiting the formation of pollen. That there is a difference between the degree of sterility conveyed through the egg and that conveyed through the pollen to the next generation is suggested by Salaman and Lesley (12). Clark (3) distinguished four different types of sterility in the tuber-bearing *Solanums*.

TABLE II.

Classification of the hybrid progenies with regard to flower colour.

Parentage	Total no. of seedlings	Flowering seedlings			
		Total no.	No. white	No. reddish purple	No. bluish purple
<i>White × White</i>					
Epicure × Flourball	71	21	21	0	0
British Queen × Flourball	51	19	19	0	0
Kerr's Pink × Flourball	75	28	28	0	0
Great Scot × 993 (a) (4)	62	28	28	0	0
Kerr's Pink × Bell	311	31	25	2	4
Great Scot × Bell	288	25	16	9	0
Flourball × Glassel Beauty	28	4	2	2	0
<i>Coloured × White</i>					
Golden Wonder × 800 (2)	9	5	5	0	0
Up-to-date × Flourball	57	18	15	3	0
Golden Wonder × Glassel Beauty	56	28	15	11	2
<i>White × Coloured</i>					
Kerr's Pink × 39 (15)	52	25	14	11	0

The majority of the parents of the hybrid progenies shown in Table II possess white flowers but apparently they are not all alike in genetic constitution. Four progenies containing no coloured-flowered seedlings were derived from the white-flowered parents Flourball, Epicure, British Queen, Kerr's Pink, Great Scot, and 993 (a) (4). Two of these varieties, viz. Kerr's Pink and Great Scot, mated with another white-flowered variety Bell, produced both coloured- and white-flowered seedlings, but in significantly different proportions. This indicates that Bell is a heterozygous white, and also that Kerr's Pink and Great Scot possess different genetic constitutions signifying that both cannot be pure whites.

Flourball, commonly known as a white-flowered variety, is really a "tinted white" since it possesses a small amount of pigment on the under-surface of the petal. It should, therefore, according to Salaman's hypothesis, produce, on crossing with a pure white, a certain number of

offspring bearing heliotrope-coloured flowers. No coloured flowers, however, appeared in these experiments. Results of five hybrid progenies may be mentioned, derived from Flourball as ♂ parent in each case, and Epicure A, Epicure B, British Queen, Kerr's Pink and Ally as ♀ parents respectively. Out of a total of 449 seedlings, 119 produced flowers, all of which were similar to one or other of the parents in respect of flower colour. A few "tinted white" flowers appeared but no heliotrope ones.

Flourball, however, when mated with Glassel Beauty (white-flowered) gave equal proportions of white and coloured flowers in the offspring. Glassel Beauty is therefore probably a heterozygous white.

It appears then that "tinted white" × "pure white" gives tinted white and pure white, and that "tinted white" × "heterozygous white" gives coloured and white, the latter probably including pure whites, tinted whites and heterozygous whites.

Of the two families derived from Great Scot × Bell and Great Scot × 993 (a) (4) (all white-flowered) the former contained both coloured and white flowers in the proportion of 9 : 16 respectively, while the latter produced nothing but white flowers. The ♀ parents being the same in both cases, the difference in results must be due to differences in the hereditary factors of the pollen parents. The variety Bell is, therefore, apparently heterozygous for flower colour, while seedling 993 (a) (4) at least shows no signs of heterozygosity in that respect.

None of the progenies derived from a coloured variety crossed with a white-flowered one, or *vice versa*, contained a majority of coloured-flowered seedlings. From the evidence, this occurrence seems to be due to the parents possessing heterozygous constitutions for flower colour.

In potato hybridisation it is invariably found that a fair proportion of the seedlings either do not produce flowers which reach maturity, or do so only sparingly. The proportion may vary considerably as, for example, in the two related families derived from Great Scot × Bell and Great Scot × 993 (a) (4) (see Table III). The former produced 8.68 per

TABLE III.

Comparisons of flower colour, etc., of three hybrid progenies.

Parentage (all white-flowered)	No. of plants in progeny	% of flower-bearing seedlings	% of white-flowered seedlings	% of coloured-flowered seedlings	% of self-fertile seedlings (natural)
A. Great Scot × Bell	288	8.6	64	36	0
B. Great Scot × 993 (a) (4)	62	45.1	100	0	75
C. Kerr's Pink × Bell	311	9.96	80.64	19.36	0

cent. flower-bearing seedlings, all self-sterile, and the latter 45.16 per cent. of which 75 per cent. were self-fertile (natural).

These plants were tested over two years since first-year seedlings cannot be relied upon to exhibit their full flowering propensities. Bell has apparently a tendency to produce few flowering offspring, and those which did flower were in this instance self-sterile. A further example of the effect of Bell is seen in the family derived from Kerr's Pink and Bell where 31 plants out of 311, or less than 10 per cent., succeeded in producing flowers in a test lasting two years. Here, again, all were self-sterile. The two varieties, Golden Wonder and Glassel Beauty, when crossed produced 50 per cent. of flowering seedlings of which only a few were naturally self-fertile.

The ♂ parents, Bell and 993 (a) (4) (Table III) exhibit wide differences in constitution although both are naturally self-fertile. A suggested difference between the degree of ♂ sterility conveyed through the egg and that through the pollen to the next generation (Salaman and Lesley(12)), and the distinguishing of more than one type of sterility by Clark(3), point towards a complex inheritance of that character which consequently cannot readily be explained in terms of single factors.

The abscission of flowers is considerably influenced by environment, but appears to be controlled to some extent by genetic factors also. There are different varieties which, although apparently possessing equal free-flowering capacities in themselves, are widely different in constitution for flower production. Scarcity of flowers may be due to a dominant factor inhibiting their production. Were this factor eliminated by inbreeding there would be no difficulty arising through lack of flowers in proceeding through an indefinite number of generations. This in itself, however, is insufficient since there is practically no correlation between flower production and self-fertility. Both free-flowering sterile plants and scanty flowering self-fertile plants are common. On the other hand, once a free-flowering self-fertile line is established it should remain so through an indefinite number of selfed generations.

TUBER COLOUR.

A wide range of tuber colour exists in the potato. Practically all the shades of reddish purple and bluish purple have been found, ranging from just a slight trace of reddish pigment at the one extreme to a deep bluish purple at the other. In addition to these self-coloured types there are others showing colour on a part of the tuber only, the remainder being uncoloured. Further, there are tubers showing individually more

than one shade of colour. The ground colour may be pale but splashed with darker patches, or the ground colour may be reddish purple with bluish purple markings on it. There is also another type which does not produce colour in the absence of light but which, on exposure to light, produces an appreciable amount of pigment. The intensity of pigmentation may increase with prolonged exposure.

In the case of varieties with faintly coloured tubers, Kelly (6) suggests the existence of a factor restricting the production of colour as found in "Red McCormick." Uniform distribution of colour over the tuber surface is introduced by a factor **S** (Salaman (9)). Collins (4), working with the King Edward variety, says that parti-colour in that variety depends upon a definite factor for which it is heterozygous. He suggests that it behaves as a dominant to recessive white and as recessive to full colour. Production of colour in the eye of the tuber, according to Salaman (9) and Asseyeva (1), is dependent upon still another factor.

Distribution of colour in the tuber will be disregarded in this discussion. In classifying tubers according to colour three groups will be used, viz. white, reddish purple and bluish purple.

Salaman considers that reddish colour in potato tubers is controlled by two factors, **D** and **R**, which independently produce no colour. **D** is a basic factor necessary for colour production and **R** is a reddening factor. A further factor **P** modifies **R**, producing a purple colour. Stuart (13), on the other hand, from a study of the hybrid progenies of

TABLE IV.

Classification of hybrid progenies with regard to tuber colour.

Parentage	Total no. of seedlings	Tuber colour		
		No. white	No. reddish purple	No. bluish purple
<i>White × White</i>				
Great Scot × Bell	288	284	4	0
Golden Wonder × 800 (2)	53	51	2	0
Great Scot × 993 (a) (4)	62	50	12	0
<i>White × Reddish Purple</i>				
British Queen × Flourball	51	26	25	0
Up-to-date × Flourball	57	29	28	0
Golden Wonder × Glassel Beauty	136	62	39	35
<i>Reddish Purple × White</i>				
Kerr's Pink × Bell	311	202	109	0
Kerr's Pink × 39 (15)	52	26	26	0
<i>Reddish Purple × Reddish Purple</i>				
Kerr's Pink × Flourball	75	30	45	0
Flourball × Glassel Beauty	28	11	17	0
Epicure × Flourball	71	24	47	0

Note. Golden Wonder is herein considered as a white-tubered variety since the russet skin apparently does not affect the genetic constitution.

white and coloured parents, wherein he obtains a consistent majority of white-tubered seedlings, concludes that "white" is not a recessive character in the seedlings of the crosses he dealt with.

According to Salaman both Flourball and Glassel Beauty are heterozygous for the factors **D** and **R** and give, on selfing, a progeny in the proportion of 9 seedlings bearing coloured tubers to 7 bearing white. A similar proportion was obtained by the writer in the case of Flourball (selfed), and also in a cross between the two varieties Flourball and Glassel Beauty. The coloured tubers possessed the red pigment but not the purple, and the range extended from a medium red to a flush of pink. The family consisted of 28 seedlings, of which 17 were coloured and 11 white.

In the family of 51 seedlings derived from British Queen and Flourball, 25 bore coloured tubers and 26 white. Various shades of red were noted but none of the tubers possessed purple. Since British Queen is a white-tubered variety it must be heterozygous, possessing at least one of the factors necessary for colour. A variety with the constitution **ddRR** would give with Flourball equal numbers of coloured- and white-tubered offspring. One-half of the coloured tubers would be darker than the other half, and the whole family would be heterozygous for tuber colour. As these proportions were obtained in the above cross, British Queen may be considered to have the constitution **ddRR**.

Similar results were obtained from the cross Up-to-date and Flourball, and the constitution of Up-to-date is therefore considered to be the same as that of British Queen, viz. **ddRR**.

In the cross between Kerr's Pink and Flourball the numbers of coloured- and white-tubered offspring obtained were 45 and 30 respectively. The tubers of Kerr's Pink are pale pink in colour, and since it must contain the factors **D** and **R**, there are indications that an inhibiting factor is also present. If such a factor, called **H**, is introduced which inhibits the action of **D**, but is incompletely dominant to it in plants homozygous for **R** or **P**, then a possible explanation may be reached. Thus a plant, either homozygous or heterozygous for both **D** and **H** but homozygous for **R** or **P**, would produce a trace of colour, herein termed a flush. On this basis Kerr's Pink may be **DDRrHh**, and when this variety is crossed with Flourball, **DdRrhh**, coloured seedlings and white seedlings in the proportion, of 40 to 24 respectively should be produced. •

Epicure, which develops a red flush on exposure to light, may be described as possessing the colour factors **DdRRHh**. Since the results

obtained by mating it with Flourball are comparable with those of Kerr's Pink and Flourball, and since Epicure possesses much less colour than Kerr's Pink, it must be heterozygous for **D** and homozygous for **R** to give the necessary proportion on the assumed basis of factor relationship. The factorial constitution **DdRRHh** stands for a flush of red, and on mating a plant of this type with one of the **DdRrhh** type (*e.g.* Flourball) a proportion of 40 plants coloured to 24 plants white should be obtained. In the cross between Epicure and Flourball the proportion of 47 coloured to 24 white-tubered seedlings was obtained, and is a close approximation to the theoretical expectation. In breeding work, therefore, Epicure should be considered as a coloured variety.

Kerr's Pink crossed with Bell gave a family of 311 seedlings, of which 109 bore coloured and 202 white tubers. This proportion bears a close resemblance to a 24:40 ratio. The tuber colour ranged from medium red to a pink flush. If it is assumed that Bell can be represented factorially by **Ddrrhh**, it should give, on mating with Kerr's Pink, **DDRrHh**, 24 coloured to 40 white.

The seedling 39 (15) gave a higher proportion of coloured-tubered offspring than Bell when mated to Kerr's Pink, *viz.* 26 coloured to 26 white. The factorial constitution of 39 (15) may therefore be described as **DDrrhh**.

In the family of 288 seedlings derived from Great Scot and Bell, four showed a trace of colour in the eyes of the tubers. From other breeding results it would appear that Great Scot when mated with Bell should give nothing but white-tubered offspring. The reason for the occurrence of four plants displaying slight colour in the eyes of the tuber is obscure, but it is possible they may have arisen through the interaction of factors for eye colour. If such is the case there is this and other evidence in favour of the factorial constitution, as regards the tuber colour, of Great Scot being described as **DDrrhh**.

Great Scot mated with seedling 993 (a) (4) produced 62 plants, of which 12 were coloured and 50 white. This is an approximation to a 1:3 ratio. The coloured tubers were all flushed pink. The results were similar to those obtained from a family derived from British Queen and 993 (a) (4). With Great Scot bearing the factors **DDrrhh**, British Queen **ddRRhh** and 993 (a) (4) **DdRrHH** the above results would be obtained.

Before considering the hybrids from Golden Wonder it may be of interest to quote the results obtained when that variety was self-fertilised. The family of 193 seedlings showed a great variety of colour,

ranging from deep purple through various shades of red to white. Classification was rendered difficult owing to the different colours merging into one another, but an attempt was made to classify the plants into three groups. In this classification 47 were "reddish," 47 "purplish" and 99 white. The other factor, **P**, apparently comes into play here. It appears that this factor not only modifies **R** but is capable of producing a purple colour in combination with **D**.

From these results it is assumed that for tuber colour the genetic constitution of Golden Wonder is **DdRrPpHh**, that is, it is heterozygous for all the factors concerned. This variety on selfing should, on this hypothesis, give a family of seedlings in the ratio of 110 coloured to 146 white-tubered plants. Of the coloured seedlings, 40 should contain more "red" than "purple," 40 more "purple" than "red," and 30 equal proportions of "red" and "purple" factors, and would be represented in 34 different genotypes. There would be 47 different genotypes among the white-tubered offspring, and only 1 in 256 would be homozygous for all the recessive factors.

Golden Wonder crossed with Glassel Beauty produced a family of 136 plants, of which 74 bore coloured tubers and 62 white. 39 of the coloured progeny were reddish and the remaining 35 purplish. The coloured types ranged from pale red to deep purple. If the genetic constitutions of Golden Wonder and Glassel Beauty are **DdRrPpHh** and **DdRrpphh** respectively, a 128:128 ratio of coloured and white tubers should be obtained. The actual ratio obtained agrees fairly well with expectation. Salaman(10) and Kelly(6) suggest the possibility of a disproportionate mortality of "creams" in certain families, and the slightly smaller proportion of white-tubered seedlings in this case may be similarly accounted for. Theoretically, of the 128 coloured seedlings, 56 should be red, 56 red with purple and 16 purple only. There should be 17 different genotypes among the coloured offspring and 19 among the white. One plant in 64 should be recessive for all the factors concerned.

Golden Wonder crossed with 800 (2) produced 51 white-tubered and 2 pink-flushed offspring. This result points to the absence of colour-producing factors and the presence of an inhibiting factor in the seedling 800 (2), and its genetic constitution may be described as **ddRrppHH**. It should give, on crossing with a variety represented factorially by **DdRrPpHh**, 8 red flushed and 8 reddish purple flushed individuals to 240 white, *i.e.* a ratio of 1 flushed to 15 white.

TUBER FLESH COLOUR.

Comparatively little is known of the inheritance of flesh colour in potato tubers. A disturbing element is occasionally found in the presence of anthocyanin pigment in the flesh where it may affect the whole or only part of the internal colour of the tuber. The presence of such pigment is often associated with skin colour, and is found in such varieties as Flourball and Congo, but pigmentation has been observed by Wilson (14) in white-skinned seedlings of a hybrid progeny, and also by the writer in immature white-skinned seedlings of an inbred family. A small proportion of the seedlings derived from Kerr's Pink \times Flourball and British Queen \times Flourball possessed tubers with pigmented flesh, but in every case the skin of the tuber was coloured. In making comparisons of flesh colour, the shade of white or yellow is considered apart from the infusion of any anthocyanin pigment.

In the ordinary commercial varieties the colour ranges from white to yellow. Salaman (9) considers that flesh colour is controlled by a single pair of factors representing deep yellow and white, that deep yellow is dominant and white recessive, that both breed true, and that the heterozygous form is a variable but pale shade of yellow. In ordinary commercial varieties deep yellows and pure whites are seldom found. Pale yellows vary from one extreme to the other, and difficulty is experienced in classification since no definite line of demarcation can be readily fixed.

TABLE V.

Classification of hybrid progenies with regard to flesh colour.

Parentage	Total no. of seedlings	No. white	No. pale yellow	No. yellow
<i>White \times White</i>				
Kerr's Pink \times Flourball	75	66	9	0
Up-to-date \times Flourball	57	44	13	0
Golden Wonder \times 800 (2)	9	5	4	0
Epicure \times Flourball	71	32	38	1
Golden Wonder \times Glassel Beauty	43	11	27	5
Flourball \times Glassel Beauty	28	5	23	0
British Queen \times Flourball	51	3	47	1
<i>White \times Pale Yellow</i>				
Kerr's Pink \times Bell	311	173	133	5
Great Scot \times 993 (a) (4)	62	19	43	0
Great Scot \times Bell	238	71	187	30
Kerr's Pink \times 39 (15)	47	14	12	21

Results of the hybrid progenies with regard to flesh colour are shown in Table V. It appears that the majority, if not all, of the varieties used in the matings are heterozygous for flesh colour. Whites when inter-

crossed should, if pure, throw nothing but whites; but apparently from varieties commercially known as whites, there is a greater likelihood of obtaining a majority of pale yellows or hybrid intermediates than a majority of whites. The commercial description of varieties cannot be taken as indicative of their genotypic nature. Pure whites, being recessive, are probably scarce, and the parent varieties used in the experiments were probably hybrids; but many were so very pale in flesh colour as to appear white. The matings of so-called white-fleshed varieties produced widely different results and three families exhibited a small proportion of the dominant yellow type.

In every case the families were the offspring either of two white-fleshed varieties or of a white-fleshed and a pale yellow-fleshed variety, and consequently yellow-fleshed seedlings were seldom found. Seedling 39 (15) appears to be genetically the deepest yellow in the series, since it produced the highest proportion of yellow-fleshed plants when crossed with the white-fleshed variety Kerr's Pink.

The various distinct ratios obtained and the wide range of hybrid forms suggest the presence of more than one pair of factors controlling the inheritance of flesh colour.

TUBER SHAPE.

An explanation of the heredity of tuber shape is given by Salaman (9,10), who says that shape, in the main, is controlled by one pair of factors, "long" and "short." Long is dominant to short but sometimes not completely. Both types breed true. The hybrid form is kidney. This gives three well-defined shapes and the numerous remaining shapes are modifications due, in some cases, to further qualifying hereditary factors, and in others to environmental influences. A modificatory factor converts "rounds" to "pebbles." These "pebbles" when crossed with the hybrid form, kidney, give a high percentage of kidneys as well as 25 per cent. pure rounds. The variety, Flourball, is not a true round, since on selfing it gives longs and rounds in the proportion of 3:1 respectively. Another form of long is the cylindrical, of which Congo is a pure type. British Queen is a short form of cylindrical.

A classification of tuber shapes is given by McIntosh (7) wherein all shapes are either rounds or longs, the latter including ovals (short and long), pear-shaped and pointed-oval types. The dominant long referred to by Salaman is in this classification termed "long oval."

For simplicity tuber shapes are herein grouped under four headings, viz. Round, Oval, Kidney and Long, and these descriptions refer more

to the relation of length and breadth than to any individual peculiarity of shape.

In Table VI it is seen that numerous shapes can be obtained from intercrossing round-tubered varieties. In many respects the results obtained are comparable. There are present in each progeny, round, oval and kidney-shaped seedlings, and in every case the figures show that ovals were in the majority and kidneys in the minority. Flourball is common in the parentage of three families showing similar proportions, consequently the other parents concerned, viz. Glassel Beauty, Kerr's Pink and Epicure can be considered approximately similar genotypes.

TABLE VI.

Classification of hybrid progenies with regard to tuber shape.

Parentage	Total no. of seedlings	No. round	No. oval	No. kidney	No. long
<i>Round × Round</i>					
Kerr's Pink × Bell	311	147	148	14	2
Flourball × Glassel Beauty	28	9	17	2	0
Kerr's Pink × Flourball	75	18	51	6	0
Great Scot × Bell	288	65	201	22	0
Epicure × Flourball	71	12	56	3	0
<i>Round × Oval</i>					
Great Scot × 993 (a) (4)	62	4	53	5	0
<i>Oval × Round</i>					
Up-to-date × Flourball	57	23	34	0	0
British Queen × Flourball	51	6	33	10	2
<i>Round × Kidney</i>					
Kerr's Pink × 39 (15)	52	5	35	12	0
<i>Kidney × Round</i>					
Golden Wonder × Glassel Beauty	136	16	66	33	21
Golden Wonder × 800 (2)	53	0	27	21	5

Different proportions were obtained from Kerr's Pink × Flourball and Kerr's Pink × Bell. The former cross exhibited a large proportion of seedlings bearing oval-shaped tubers, almost three times as many ovals as rounds, while the latter gave practically equal numbers of rounds and ovals. In both cases a small proportion of kidneys was found, but only in the progeny of Kerr's Pink × Bell did longs appear. It is probable that longs would also appear in the progeny of Kerr's Pink × Flourball if a comparably large number were grown, since the average tuber shape in this family possesses a relatively longer axis than that of the family derived from Kerr's Pink × Bell. Bell appears to be less heterozygous for the factors for round-tuber shape than Flourball, and although both are popularly termed round-tubered varieties, the latter breeds more as an oval than as a round.

Kerr's Pink and Great Scot, as ♀ parents, were crossed with Bell.

From the figures it is obvious that Kerr's Pink breeds truer to round than Great Scot. It throws a higher percentage of rounds and a lower percentage of ovals and kidneys than Great Scot.

In the class formed by mating an oval with a round there are two progenies showing diverse results, although the parents, Up-to-date \times Flourball, and British Queen \times Flourball, are comparable. The constitutional difference lies in the ♀ parents, since the ♂ parent is the same in both cases. Up-to-date \times Flourball has a tendency to produce ovals and rounds, and British Queen \times Flourball ovals and kidneys. The tubers of both Up-to-date and British Queen are classed as oval, but within that class British Queen tubers possess a slightly longer axis than those of Up-to-date. This difference is emphasised by the results obtained from the progenies of these two varieties when crossed with Flourball, and it indicates that British Queen is genotypically allied to the kidney rather than to the oval-tubered type.

Two progenies derived from Golden Wonder \times Glassel Beauty and Golden Wonder \times 800 (2) respectively are in each case hybrid plants of a kidney variety crossed with a round. The results suggest that Glassel Beauty, although rather flat, is genetically a less heterozygous round than 800 (2) since the latter variety produced no round tubers in the mating. The appearance of long-tubered plants which transgress the range of the parental type may be attributed to the heterozygous nature of the parents and particularly to that of the ♀ parent.

In the 11 matings given in Table VI there was obtained in each case a majority of ovals. Five families, derived from round-tubered parents and including altogether 773 plants, gave over 61 per cent. of oval-tubered offspring. In each of the families a small proportion of kidney-shaped tubers appeared, attaining on an average just over 6 per cent. With the exception of two long-tubered seedlings which appeared in a family of 311 derived from Kerr's Pink \times Bell, the remainder, about 32.4 per cent. were round. Obviously the majority, if not all, of these round-tubered parents are heterozygous for tuber shape, and various kinds are involved. Two different kinds of ovals have been demonstrated in Up-to-date and British Queen.

The inheritance of tuber shape is genetically complex, and in most modern varieties this character probably exists in a heterozygous condition. The shapes are so numerous and the differences so slight that it is hardly possible to make an accurate general classification. The classification used in the foregoing notes is purely arbitrary and it is admitted that it is sometimes difficult to decide to which of two classes a plant

actually belongs. Between the two extremes "long" and "round" there exists a continuous series of intermediate shapes, including a range of flat and cylindrical and irregularly pointed tuber types. The range of types as found in one progeny is shown in Fig. 1, and shape is represented by ratio of length to breadth.

The graph was obtained by plotting the ratio, maximum length/maximum breadth, against the frequency, and illustrates the range of types together with their frequency in a family of 136 F_1 seedlings derived from Golden Wonder and Glassel Beauty. The ratio with the maximum frequency lies between the ratios of the parents, but closer to

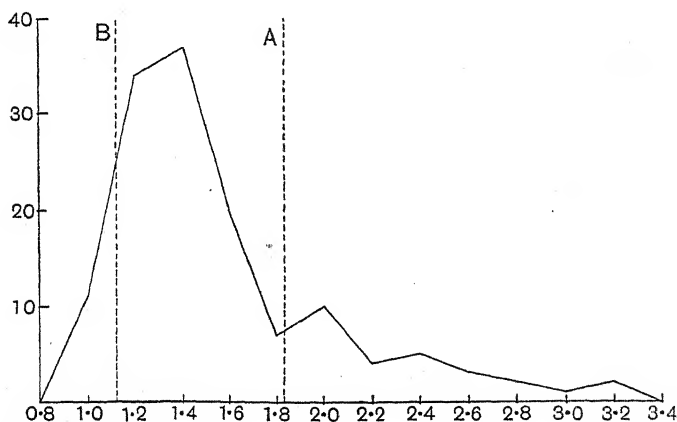


Fig. 1.

that of Glassel Beauty (B) than to that of Golden Wonder (A). Transgression occurs in both directions but it is more pronounced in the higher ratios.

Tuber shape, from the point of view of the relation between length and breadth alone, appears to be controlled by more than one pair of factors. Peculiarities of shape, such as the pear-shaped type, introduce other factors which may function as modifiers. Such modifying factors appear to be numerous, and it seems probable that the number of factors controlling tuber shape may vary in different varieties.

EYE DEPTH.

The depth of eyes, except in the two extremes, is difficult to estimate accurately on account of its variable nature. It is affected to a great extent by environmental conditions. Eyes tend to be deeper in tubers grown on wet clayey soil, and shallower in those grown in dry sandy soils.

Again, the effect of second growth is often sufficient to make normally deep eyes appear shallow or even superficial. Apart from these conditions classification according to depth of eyes is, at the best, merely the expression of an opinion which cannot readily be arrived at by actual measurements.

Eye character according to Salaman (9) is inherited on a single factor basis. Deep eyes breed true, but only in some cases do fleet eyes breed true. The latter character is incompletely dominant and the hybrid between deep and fleet is more fleet than deep.

TABLE VII.

Classification of hybrid progenies with regard to eye depth.

Parentage	Total no. of seedlings	No. shallow	No. medium	No. deep
<i>Shallow × Shallow</i>				
Golden Wonder × Glassel Beauty	136	77	53	6
<i>Shallow × Medium</i>				
British Queen × Flourball	51	39	12	0
Golden Wonder × 800 (2)	53	39	14	0
Up-to-date × Flourball	57	15	38	4
<i>Medium × Shallow</i>				
Flourball × Glassel Beauty	28	21	7	0
Great Scot × 993 (a) (4)	62	39	21	2
Kerr's Pink × 39 (15)	52	31	20	1
<i>Medium × Medium</i>				
Great Scot × Bell	288	204	75	9
Kerr's Pink × Flourball	75	39	36	0
Kerr's Pink × Bell	311	87	199	25
<i>Deep × Medium</i>				
Epicure × Flourball	71	38	32	1

The crossing of two shallow-eyed parents, Golden Wonder and Glassel Beauty, gave an unexpectedly high proportion of medium- and deep-eyed seedlings. Only 56.6 per cent. of shallow-eyed types were found, which indicates a heterozygous constitution in at least one of the parents. Golden Wonder has in other experiments proved itself to be heterozygous for eye depth. The progeny of Golden Wonder × 800 (2) contained a higher percentage of shallow-eyed plants than that of Golden Wonder × Glassel Beauty, although the eyes of 800 (2) are of medium depth. Glassel Beauty may therefore be regarded as heterozygous for eye depth.

Of the two families derived from British Queen × Flourball and Up-to-date × Flourball, the former gave a comparatively high percentage of shallow-eyed plants, while in the latter the percentage was comparatively low. The difference lies in the female parents and indicates that Up-to-date breeds deeper-eyed types than British Queen.

With the exception of one instance the mating of medium- and

shallow-eyed parents or *vice versa* gave, in each case, a majority of seedlings with shallow-eyed tubers.

Comparisons between the hybrid families of medium-eyed parents demonstrate that Great Scot tends to give more shallow-eyed seedlings than Kerr's Pink, and Flourball more than Bell.

The hybrids of medium- and deep-eyed varieties as derived from Epicure and Flourball comprise comparatively large numbers of shallow-eyed seedlings. The small percentage of really deep-eyed plants obtained is consistent with the view that deep eyes are recessive.

From the evidence obtained from the progeny of Golden Wonder \times Glassel Beauty shallow eyes sometimes do not breed true. Various families in the F_2 generation have been found to breed true for shallow eyes. This is in agreement with Salaman's views (9) on shallow-eyed tuber types.

It seems highly probable that eye depth is controlled by genetic factors which represent the extremes of types, and that all intermediates between true deep-eyed and true shallow-eyed are heterozygous. The majority of the parents in the hybrid progenies just described are undoubtedly heterozygous. There is a distinct tendency for hybrid progenies to give a large proportion of forms intermediate between the parent types, and transgression frequently occurs to a limited extent.

SUMMARY.

Various hybrid progenies are described and compared with reference to several characters, viz. flowers (flower colour and flowering capacity), tuber colour, tuber flesh colour, tuber shape and eye depth.

Various genetically different white-flowered varieties are noted, and coloured flowers doubtless represent numerous different genetic types. The inheritance of flower colour appears to be controlled by several factors.

Considerable hereditary differences in flowering capacity and in fertility are noted in varieties which appear to be similar for these characters.

It is suggested that the inheritance of tuber colour apart from pattern in the potato is in several varieties controlled by four factors, viz. **D**, a basic factor necessary for the production of colour alone; **R**, a factor which in conjunction with **D** produces a red colour; **P**, a factor which in conjunction with **D** produces a purple colour, and in conjunction with **D** and **R** an intermediate reddish purple colour; and **H**, an inhibiting

factor incompletely dominant to the factor **D** in the presence of homozygous factors **R** or **P**.

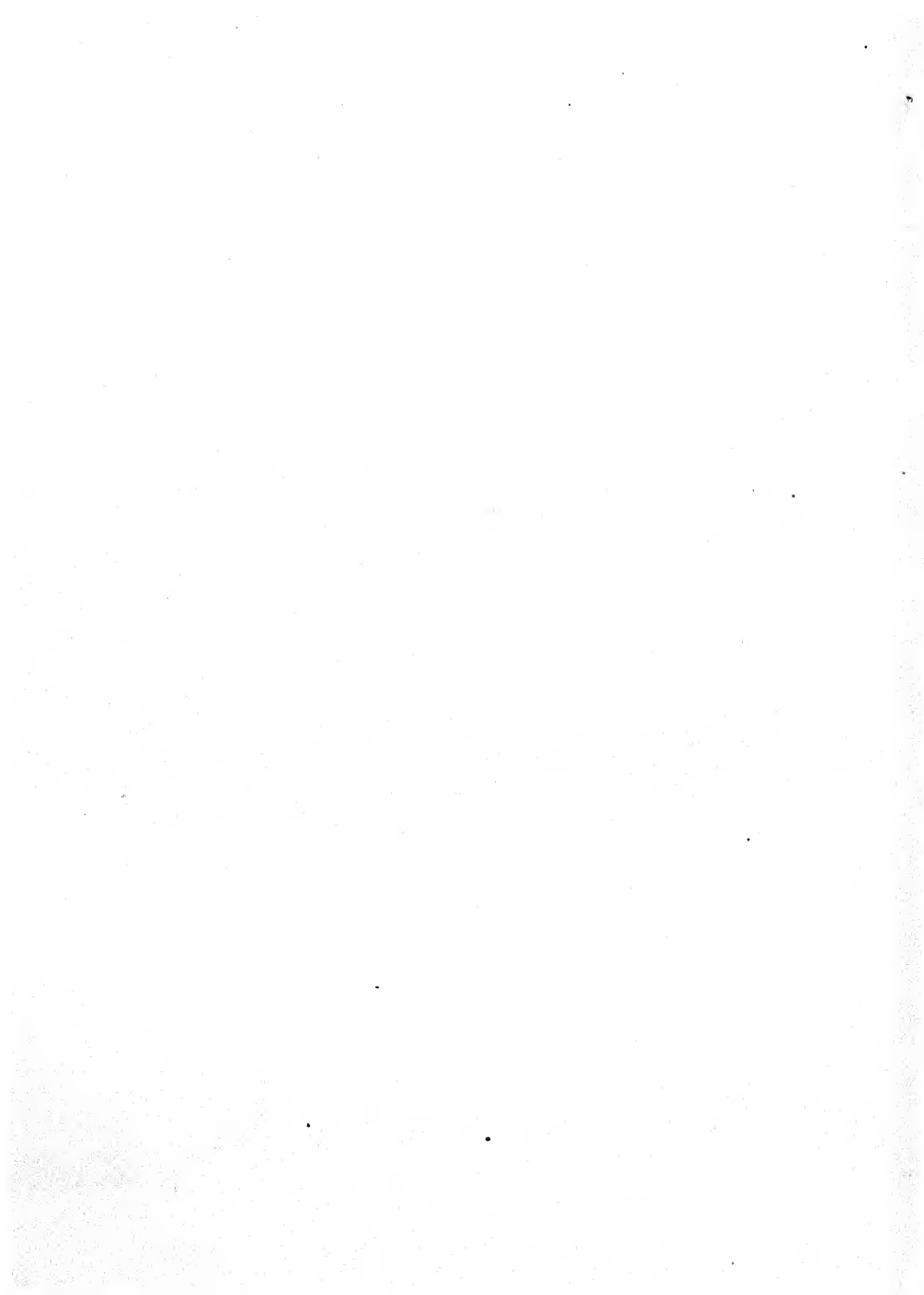
Tuber flesh colour is controlled by genetic factors and probably by more than one pair. So-called white-fleshed varieties are, in many cases, hybrid intermediates.

Tuber shapes include practically all the intermediates between round and long considered from the point of view of the ratio length/breadth. Many so-called round-tubered varieties appear to be heterozygous. In hybrid progenies, transgression frequently occurs. Various factors are involved and the number probably varies in different varieties.

The depth of eyes in the tuber appears to be controlled by genetic factors, probably representing the extremes, viz. deep-eyed and fleet-eyed. The parents discussed are probably all hybrids. Transgression is found to occur in certain hybrid progenies.

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THE INHERITANCE OF BUTTER-FAT PERCENTAGE IN CROSSES OF JERSEY WITH RED DANES.

BY THE LATE CHR. WRIEDT.

(With Fifteen Text-figures.)

PRACTICALLY all the economically important characteristics of our domestic animals depend upon a complicated reaction between heritable factors and the conditions under which the animals live. In the case of the larger domestic animals in particular this fact makes a genetical analysis of the economically valuable heritable factors extremely difficult. Moreover we now know that, in addition to variations due either to genetic causes or to living conditions, there are other variations whose causes we do not know.

It is but natural then that no great progress has been made in the genetic analysis of the economically valuable characteristics of our domestic animals. Experiments on a sufficiently large scale have, practically speaking, not been made. Then too, when experiments were made, F_2 was bred and no use made of the only rational method with slow-breeding material, namely, crossing back. Besides, it is always a case of quantitative characteristics, and in Castle's experiments (1925) on length of ear and weight of rabbits the interpretation was on a false track. It was a general precept that quantitative characters practically always depended on a large number of similarly operating factors, and as the editing of the often weak genetic material on the important characters of domestic animals was generally left to persons without experimental experience, the analysis was confined to a computation of the variation coefficient for the parent types, F_1 and F_2 . If the variability in F_2 was considerably larger than in the original types and in F_1 , the conclusion was drawn that it was a case of polymeric factors.

The curves from the figures obtained were made the object of close study, even though they only comprised 30 to 40 individuals grouped in eight or nine classes. As a rule the fact that both variations due to living conditions as well as others due to non-genetic causes, were being studied was forgotten. The former is hard to excuse; the latter may be excused on the ground that several of these studies were made before it was realised that non-genetic variations of unknown cause must be taken into account. Sewall Wright's study (1920) of white spots in guinea-pigs

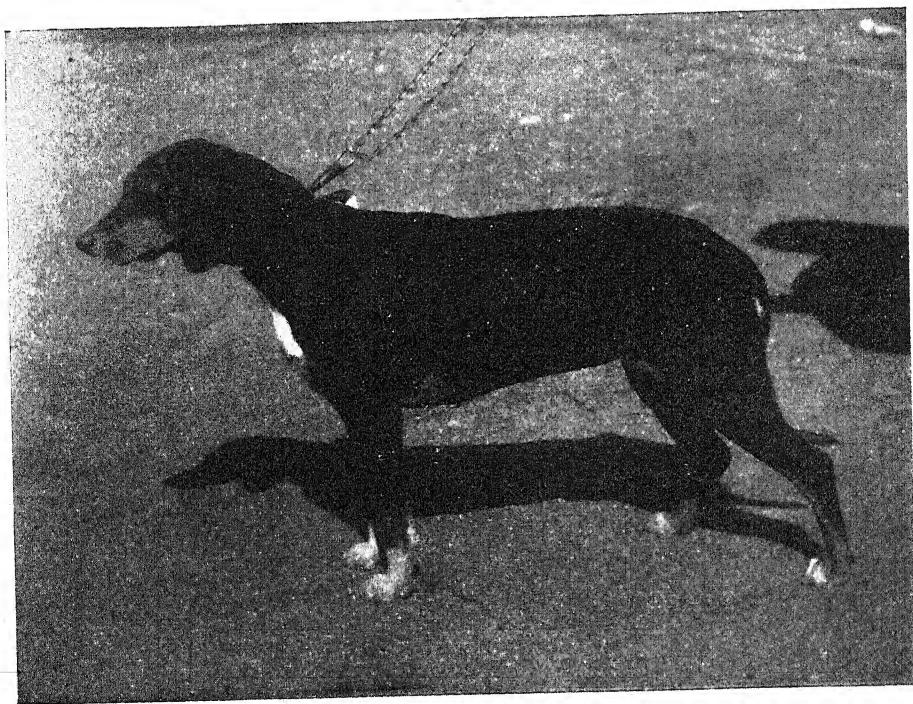


Fig. 1. Dunker hound, slightly spotted.

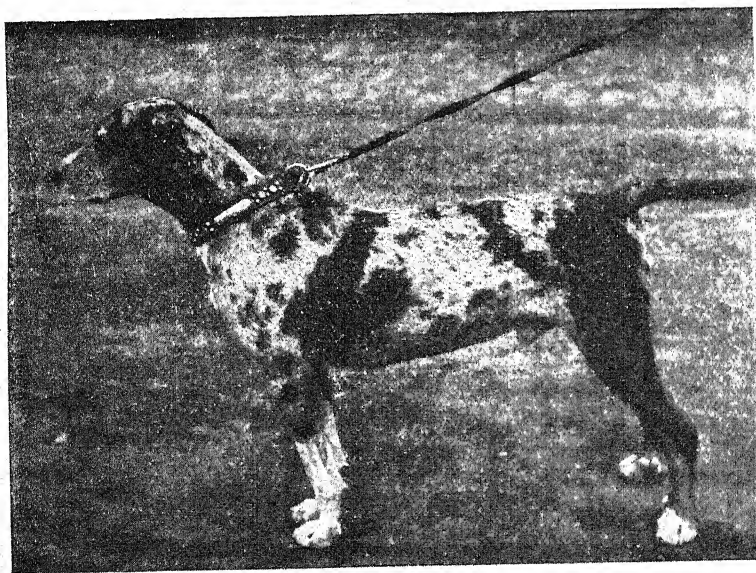


Fig. 2. Dunker hound, ordinary markings (spots).

was the first investigation demonstrating the necessity of accepting non-genetic variability which had nothing whatsoever to do with living conditions. Any one who has made experiments knows that the majority of heritable factors may vary in their phenotype. From my own experience I can cite as example the gray spots of the Norwegian Dunker hound. Heterozygotes for this factor can vary from evenly spotted over the entire body to a single spot the size of a crown piece on the neck. However heterozygotes with the single spot in the neck, when bred with

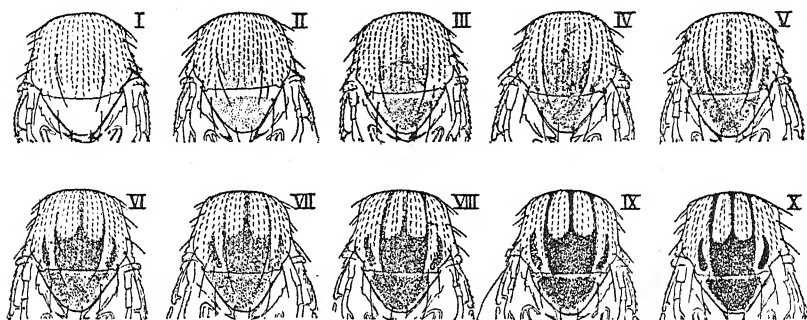


Fig. 3. The scale of grades of the trident-pattern in *Drosophila* (after Morgan and Bridges).

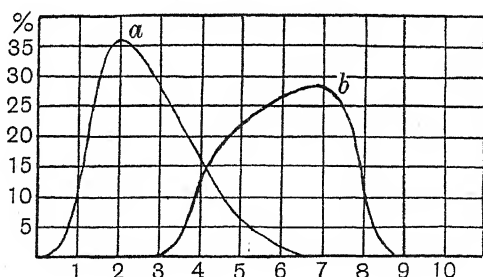


Fig. 4. Curves representing distribution of grades in stock a and stock b (redrawn from Morgan and Bridges).

non-spotted, produce puppies of which one-half are spotted exactly as the puppies of the evenly spotted parents. In a statistical analysis of the spread of spottedness in such hounds it could easily be proved that more than one similarly operating factor was concerned. Morgan and Bridges (1919) have used *Drosophila* in investigations which show exceptionally well how such misunderstandings arise. The experiment was made with the factor for a trident spot on the thorax. The spot was extremely variable, and the bounds between the spotless breed and

the spotted breed were not sharp. The animals were grouped into ten classes and the accompanying illustration shows how the division was made. In the first place both of the original types were used for pure breeding, and 1614 individuals of the type "without" and 2538 of the type "with" were produced. As shown in Fig. 4, the two types overlap. The next step was to breed an F_1 of 2587 individuals. As seen in Fig. 5,

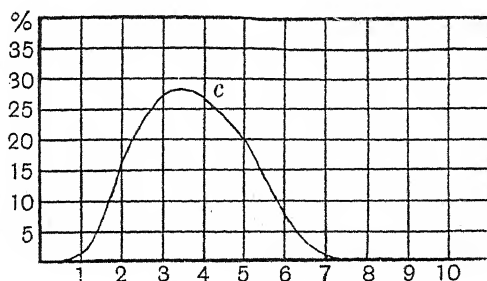


Fig. 5. Curve representing distribution of grades in F_1 (redrawn from Morgan and Bridges).

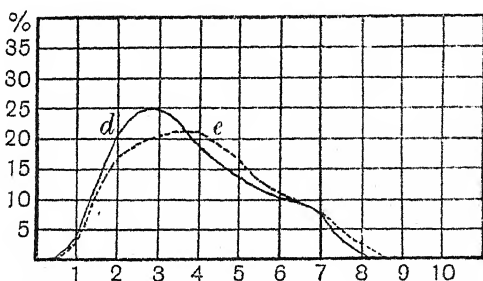


Fig. 6. Curves representing distribution of grades in F_2 ; e = expected F_2 as calculated from the observed distributions in the parental stocks and F_1 (redrawn from Morgan and Bridges).

the curve for this lies fairly closely between those of the two original types. An F_2 of 3100 individuals was then bred, illustrated by curve d , Fig. 6. Most workers would probably have drawn the conclusion that this was due to a recombination of several different factors. Morgan and Bridges (1919), however, computed how the curve would appear when the curve for F_2 was drawn on the basis of curves for both the original types and F_1 , for they believed they were dealing with a simple Mendelian segregation with the numerical proportion 1 spotted : 2 F_1 : 1 spotless. This curve is shown in curve e , Fig. 6, and we note the striking agreement there is between this curve and curve d , Fig. 6. If there had been more

factors in this experiment the curve for F_2 would have been of another type than the one computed, for there would have been fewer flies which resembled their grandparents.

This analysis shows how dangerous it is when investigations in genetics are made by persons who lack experimental experience in quantitative characters.

The genetic analyses made of butter-fat percentage in the milk of cows have suffered from the difficulties indicated above. We now know that butter-fat percentage in milk depends to a great extent on the fodder, exercise and conditions of mating of the cow. This brings about a great variability which has nothing whatever to do with heredity; and to this must be added the possibility, perhaps, indeed, the probability, of variations whose causes are unknown.

The first investigations on the heredity of high butter-fat percentage in milk were made by comparing the butter-fat content in the milk of the daughters of the bulls with that of their mothers. The earliest published investigations of this relation were made by Högström in 1906. He found differences in the various bulls in their transmission of the factor for high butter-fat content, and believed that he could prove that heredity of butter-fat content followed Galton's law. In 1911 was begun the publication of a series of investigations on the heredity of the factors for amount and butter-fat content of milk in Red Danish bulls. These investigations showed that various genetic types exist among Red Danish cattle as regards butter-fat content. This fact has been substantiated by later investigations of several other races.

Several experiments have been made to demonstrate segregation of butter-fat percentage under cross-breed analysis. These experiments are subject to the sources of error mentioned above; for they are based on the inexpedient F_2 method of breeding, and the deductions are drawn from too small a number of animals. In addition they show considerable ignorance of the original material used in the cross-breeding analysis.

In 1924, while investigating a cross between Jerseys and West Country red cattle, I noted that the factor for high butter-fat content of Jersey cattle seemed to segregate in a comparatively simple way; and I began to look about for material with which to analyse the butter-fat content in milk of this particular breed. The material at hand at Jæren, Norway, where my preliminary investigations were made, was not suitable for drawing conclusions. The herds were small and the fodder conditions varied greatly.

Through collaboration with Prof. Lars Frederiksen, of the Royal

Veterinary and Agricultural College, Copenhagen, Denmark, I was given the opportunity of revising the cross-breeding experiments with Jersey and Red Danish cattle made on a very large scale on Count Ahlefeldt Laurvigen's estates on Langeland. These experiments were begun in 1906 and are still going on. The records have been made under the supervision of Mr Henriksen, manager of the estate, who has taken every precaution to render the material as trustworthy as possible. The material was found to be very comprehensive, for, in order to make the investigations complete, I included the Red Danish mother cows, their Red Danish daughters, the pure-bred Jersey cows from the bull used in cross-breeding, the daughter cows of the Red Danish bulls used in crossing back, and, of course, all the cross-bred cows of various degrees of cross-breeding. The investigations comprised a total of 1175 cows.

After various computations and conferences with Prof. Frederiksen, I decided to choose the average of the butter-fat content for the third to the sixth month of the lactation period as basis. The lactation periods following casting are omitted. The method has, of course, several sources of error, and two of these are so grave that we have investigated them. The first is the effect of the age of the cow on the percentage of butter-fat in her milk. In studying this 28 cows were selected which had milked normally during eight lactation periods. The average butter-fat percentage for these cows is seen in the following:

	1	2	3	4	5	6	7	8
Average butter-fat percentage	4.38	4.37	4.38	4.31	4.24	4.23	4.20	4.14

This table shows the agreement between Gowen's (1924) and other investigations of the relationship between the age of the cow and the percentage of butter-fat. Since in only a very few cases do we know the amount of milk given by cows in eight lactation periods it is not worth making calculations for corrections. It is an accepted fact that the butter-fat percentage often increases when cows are turned out in the field, and soon falls again. The lactation periods in which the third to the sixth month includes the month of May will, therefore, show a slightly higher butter-fat percentage. An investigation of this relationship for 232 cows showed that the difference, when May was included, and when it was not, was 0.024. The greatest increase was 0.23 per cent. and the smallest 0.14 per cent. The difference is not sufficiently great in this case either to warrant a correction.

In undertaking an analysis of the material at hand the first step was, of course, an investigation of the butter-fat percentage in the milk of the breeds which were our starting point. We note then, that the 108 Red

Danish cows which were used in F_1 had an average butter-fat percentage in their milk of 3.40 (see Fig. 7). The variation was from 2.8 to 4.4. The variation coefficient is 8.92 ± 0.61 . To obtain a figure to express the butter-fat percentage of the Jersey cows I have taken an average of the butter-fat percentage of the Jersey daughters of the four Jersey bulls having the greatest number of F_1 daughters. The average percentage in 66 cows is 5.57, varying from 4.7 to 6.6. The variation coefficient is 7.46 ± 0.68 .

As we have seen, the two parent breeds vary greatly, and in both cases investigations of the progeny—Danish investigations in the one instance and investigations from the Maine (U.S.A.) Experiment Station in the other—have demonstrated that several heritable factors exist which affect percentage of butter-fat content in milk. Of course these conditions render a cross-breeding analysis difficult, but the difference between the two races— 2.17 ± 0.059 —and the fact that, in the case we are studying, they do not merge into each other, makes the material we have had at our disposal very valuable.

FIRST CROSS-BREED GENERATION, F_1 .

The 108 F_1 cows had an average butter-fat percentage of 4.39, varying from 3.4 to 5.5. The variation coefficient is 9.72 ± 0.66 . When we study the graphic presentation of the relation between the percentage of butter-fat content in the milk of the F_1 daughter cows and that of their mothers (cf. Fig. 7), we observe that only 1 of the 108 cows has a lower percentage than the mother, 1 has practically the same and the remaining 106 a higher butter-fat percentage. The difference varies, however, from 0.3 to 2.0. This indicates that, as already stated, we are dealing with a factor which varies, depending on conditions of living. It also shows that the genotype can vary in respect of specific factors for high butter-fat percentage in the Jersey bulls used. I have investigated this last condition, and have been unable to note that any one of the bulls used could be pointed out as especially able to increase the butter-fat percentage.

One bull produced 8 of 11 daughters with butter-fat percentage below 3.9, but this was due to the fact that he was mated with Red Danish cows with strikingly low butter-fat percentage. Another important condition to be considered is whether the one or several genetic factors for high butter-fat percentage, which are to be found in Jersey cattle, act differently when combined with factors for low or relatively high butter-fat percentage in the Red Danish cows. This condition can

be best investigated by computing the correlation coefficient between F_1 daughters and their Red Danish mothers, and then comparing the results with results obtained from other material. The correlation coefficient between the butter-fat percentage of the F_1 daughters and that of their mothers is 0.411 ± 0.08 . In the present material the

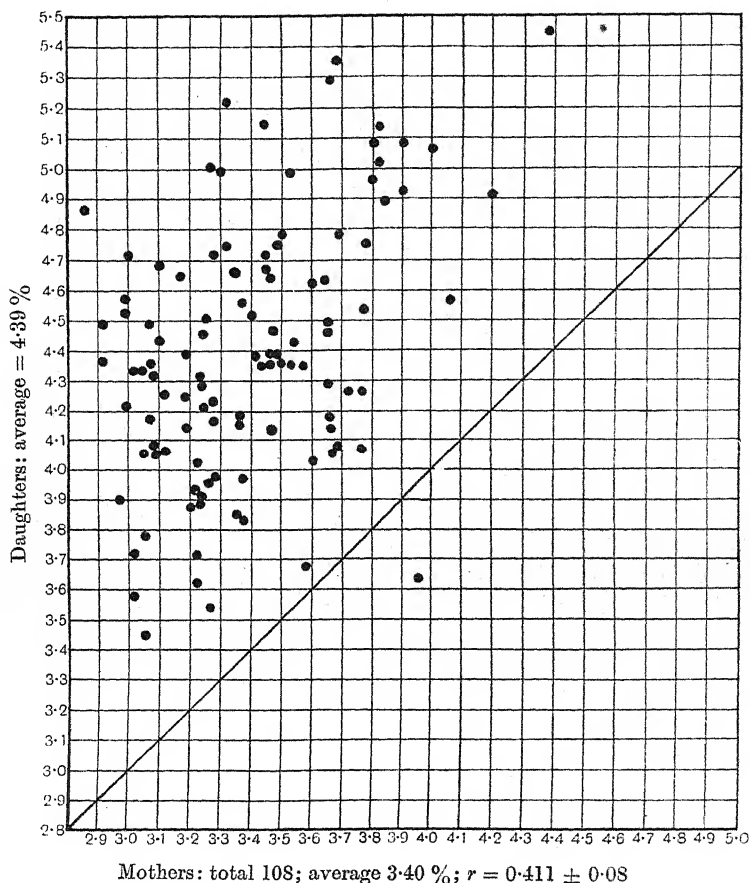
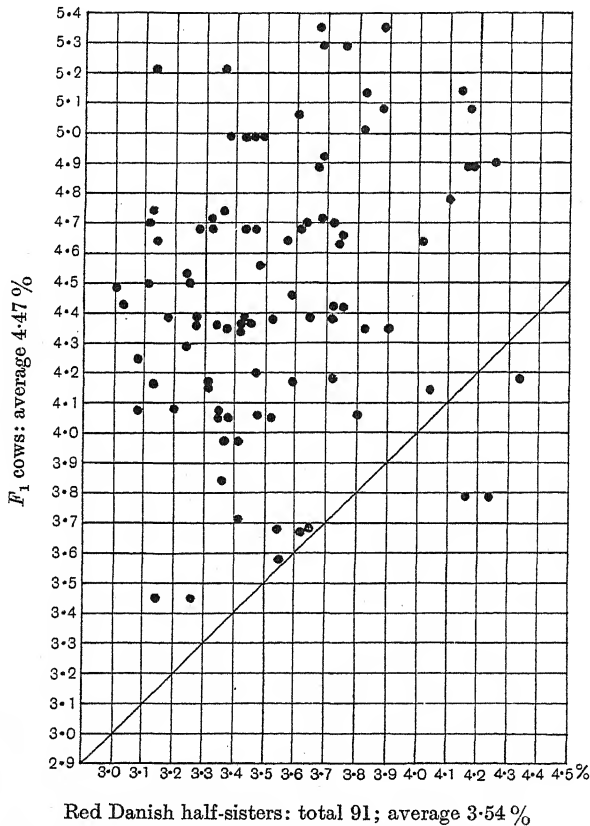


Fig. 7. Graph showing butter-fat percentage in milk of F_1 cows as compared with their mothers.

correlation coefficient for the butter-fat percentage of the Red Danish daughter cows and their mothers is 0.33 ± 0.098 , and for the Jersey daughter cows and their mothers 0.37 ± 0.11 . Using Holstein-Friesian cattle, Gowen (1924) found a correlation coefficient of 0.413 ± 0.023 . This indicates clearly that in the present cross between Jersey and Red

Danish, we cannot count on any other correlation between the butter-fat percentage of the daughter and mother cows than that found within the so-called "pure breeds." Therefore no demonstrable difference in the effect of one or more specific Jersey factors for high percentage of butter-fat content in milk, depending on whether they combine with relatively high or low butter-fat percentage in Red Danish cows, can be shown.



* Fig. 8. Graph showing butter-fat percentage in milk of F_1 cows as compared with their Red Danish half-sisters.

As a further control of the difference between F_1 and Red Danish cows, I have made a comparison between the F_1 cows and their pure Red sisters on the mother's side. There were 91 of these half-sisters (cf. Fig. 8).

From this comparison we note that the Red Danish half-sisters show

an average butter-fat percentage of 3.54, varying from 2.9 to 4.4. The variation coefficient is 8.92 ± 0.66 . The average butter-fat percentage is, then, slightly higher than that of the Red Danish mothers. The variation coefficient is the same. The F_1 cows included in this investigation showed an average butter-fat percentage of 4.47. The difference between the Red Danish half-sisters and the F_1 cows is 0.93; in other words almost the same as between the F_1 cows and their mothers, namely, 0.99. With regard to the variability in the effect of the Jersey factor we observe that F_1 cows in three instances show a lower butter-fat percentage than the Red Danish half-sisters, and in six instances the same. With regard to the difference in butter-fat percentage we note the same variation as between the F_1 daughters and their mothers. The correlation coefficient between the F_1 cows and their Red Danish half-sisters is, in this case, 0.18 ± 0.01 . This correlation coefficient is practically the same size as that found by Gowen for the half-sisters in his Holstein-Friesian material, namely, 0.173 ± 0.037 . This corresponds closely with the results for the F_1 cows and their mothers, and substantiates the conclusion, drawn from the present material, that there is no demonstrable difference in effect of the specific Jersey factors for high fat percentage, whether they combine with a factor for relatively high or low butter-fat percentage in milk of Red Danish cows or not.

CROSSING BACK TO RED DANISH.

The material affords 42 back-crosses of Red Danish bulls on F_1 cows (see Fig. 9). These cows show an average butter-fat percentage of 4.4. The average of their mothers is 4.36—that is to say, good agreement with the total F_1 . The butter-fat percentage of the daughters varies from 3.3 to 5.0. This variation extends from below the average for the Red ancestral dams to 0.6 above the average for F_1 . In the lower classes, provided one single factor only was present to cause the difference between Red Danish and Jersey, we might have expected that these would have been more largely represented; however, we must remember that in this case we are dealing with a spreading which extends over 27 classes if we include the extreme variants in Red Danish and F_1 , and we can hardly expect to find all classes represented in 42 individuals. Then too, we have constantly attempted to obtain Red Danish bulls with the best possible genotype for high butter-fat percentage, and these have been used in crossing back. Segregation of the factor for high butter-fat percentage seems to indicate that we are dealing with a primary factor which causes the difference in butter-fat

content between the Jersey and the Red Danish cows. Of the 42 cows used in crossing back, 9 are above the average for F_1 and the maximum for Red Danish. If one-half of the heterozygotes were above the average for F_1 we would expect 10.5, which agrees well with the 9 actually obtained. Of course too much weight must not be laid on such an agreement, and in this material we will not attempt to compute the number of individuals in the various classes—as done by Morgan and Bridges (1919) in their investigation of *Drosophila*—the number of classes is too large and that of individuals in the various generations too

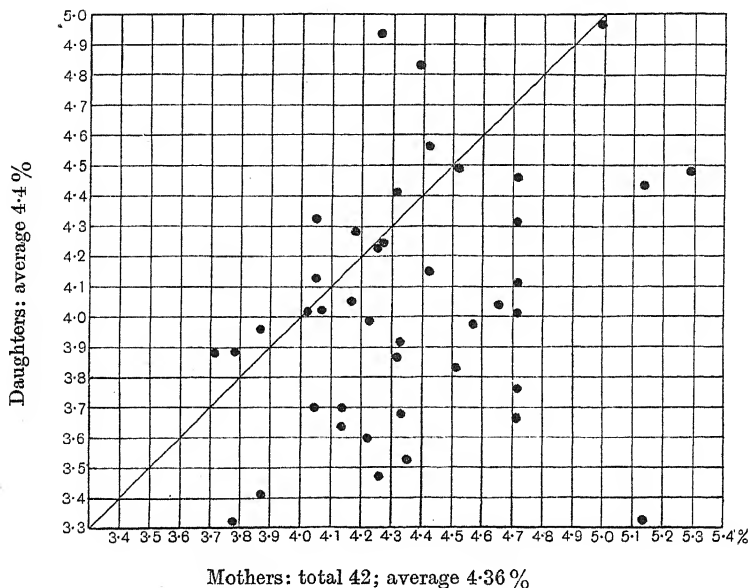


Fig. 9. Graph showing butter-fat percentage in milk of cows bred by crossing back to Red Danish as compared with their mothers.

small. With this kind of material it is impossible to procure enough individuals for reliable calculations of this sort.

When the cows used in crossing back are compared with their F_1 mothers, we find that 3 have a higher butter-fat percentage than the mothers—1 with even 0.7 per cent. higher—and that 15 have the same percentage. In short, 18 of the 42 cows used in crossing back have the same, or a slightly higher, butter-fat percentage than the F_1 cows. These 18 have mothers whose butter-fat percentage varies from 3.8 to 5.0 and they are evenly distributed among all the classes.

According to their phenotype, these 18 cows should possess the same

factors for butter-fat percentage as their mothers, that is to say, they have the specific Jersey factor for high percentage butter-fat in milk in a single dose. Of the 42 cows used in crossing back we should expect 21 of the same phenotype as the mothers—supposing that it is a question of *one* factor only. In the present material we have found this to be true of 18 cows, which agrees fairly well with our expectations.

Among the 42 cows used in crossing back it is fortunate that 17 were sired by the same bull. This bull, Holey, has also sired 46 Red Danish daughters. This material is as homogeneous as can be obtained, and is therefore interesting. As seen in Fig. 10, Holey's Red Danish daughters

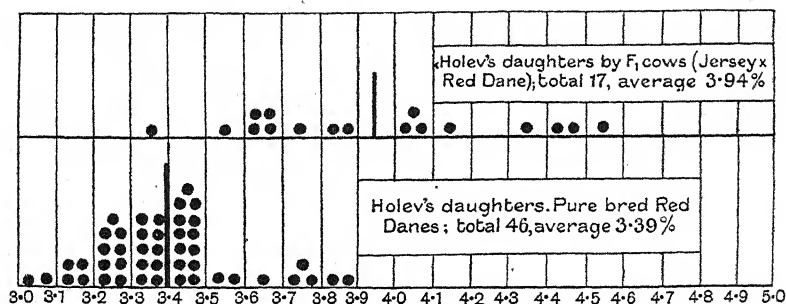


Fig. 10. Graph showing butter-fat percentage in milk of cows sired by Holey.

have a butter-fat percentage varying from 3.0 to 3.9 and the daughters of the cows used in crossing back from 3.0 to 4.6. We note from the chart that the segregation in crossing back might be used as a model if we take for granted that only *one* factor is concerned, for 9 cows have a butter-fat percentage under 3.9, and 8 have a butter-fat percentage above 4.0.

BACK-CROSSES WITH THE JERSEY.

Crossing back to the Jersey has given 49 cows (see Fig. 11). Their average butter-fat percentage is 4.82, varying from 4.0 to 5.9. The variation coefficient is 8.71 ± 0.88 . The average butter-fat percentage of their mothers is 4.24. The difference between these F_1 mothers and the total F_1 is not more than 0.15 per cent. We should therefore hardly have expected that a selection had been made of cows with low butter-fat percentage for use in cross-breeding back with Jersey. However, such was the case, for none of the 18 F_1 cows with a butter-fat percentage above 4.8 was used in crossing back, and only 6 of the 22 cows with a butter-fat percentage of 4.5–4.8 have daughters by crossing back with Jersey bulls. In spite of this selection the result of this back-cross is

a very clear segregation of the factor for high percentage of butter-fat content in milk, for there are 25 individuals with a butter-fat percentage above 4.7, the lowest of the Jersey. However, we must remember that among these—taking of course for granted that one factor only causes the difference in butter-fat percentage between Jersey and Red Danish—we may expect to find some with a single dose, for F_1 varies up to 5.5 per cent.

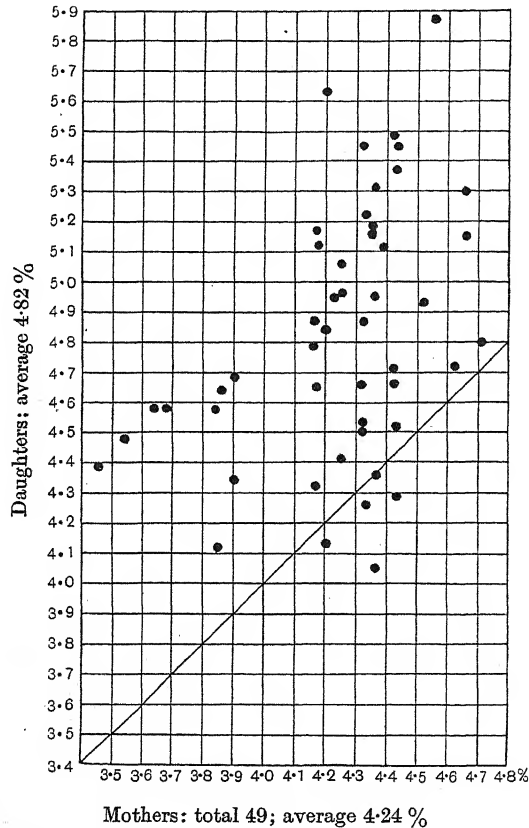


Fig. 11. Graph showing butter-fat percentage in milk of cows bred by crossing back to Jersey as compared with mothers.

In crossing back with Jersey it is strikingly evident that but few individuals have a lower or about the same butter-fat percentage as the mothers, there being but one with 0.3 less than the mother, and indeed but 13 with about the same butter-fat percentage or less than the mother. This does not agree with the results from crossing back with Red Danish.

In the present case 13 of the 49 cows have about the same butter-fat percentage as the mothers, while in crossing back with Red Danish 18 of the 42 cows had the same butter-fat percentage as the mothers. This can be partially explained by the selection of the F_1 cows used which was mentioned above. However it is probable that other causes exist. It is difficult to ignore the possibility that among some of the Jersey bulls used, in addition to the primary factor for high percentage of butter-fat content in milk, another factor is to be found which increases the butter-fat percentage, though to a far slighter extent. If this is accepted as a possibility it will also explain the small segregation in the lower classes in cross breeding back with Red Danish bulls.

COWS BRED FROM RED DANISH BULLS AND DAMS BRED BY
CROSSING BACK TO JERSEY BULLS.

The appearance of such a modifying factor seems quite probable when we consider the results of crossing Red Danish bulls with cows bred by crossing back to Jersey. We have 11 cows from this mating and their average butter-fat percentage is 4.48, while that of their mothers was 4.91.

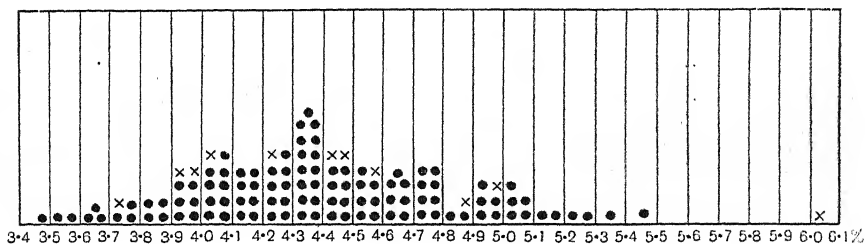


Fig. 12. Chart showing butter-fat percentage in milk of F_1 cows, and of cows bred from Red Danish bulls with dams bred by crossing back to Jersey bulls. ● = F_1 cows. x = cows bred from R.D. bulls and dams bred by crossing back to Jersey bulls.

Fig. 12, in which 11 cows are compared with the F_1 individuals, shows that 6 are above the average for F_1 , and that 1 shows a butter-fat percentage of 6.0. The remaining 5 group themselves nicely among the individuals of the lower half of F_1 . It would be unreasonable to suppose that all the cows used in crossing back had the Jersey factor in a double dose, and it conforms much better with the actual results to suppose that Jersey cattle sometimes possess a modifying factor which increases the butter-fat content.

On the other hand this result tells strongly against polymeric factors, for on this hypothesis such a distribution of even so small a number as 11 would be most unlikely.

SUMMARY OF THE RESULTS FROM F_1 AND OF CROSS-BREEDING BACK
WITH BOTH PARENTAL BREEDS.

We have now studied in detail the results from F_1 and from crossing back on both sides, and we may now consider our analysis as a whole as illustrated in Fig. 13. It shows that F_1 lies between the average figure for Red Danish and Jersey and that cross-breeding back on both sides, especially with the Jersey, leads to a distribution which best conforms to the hypothesis of one primary factor, and possibly one modifying factor. In this connection great emphasis is placed upon the fact that neither the Red Danish nor the Jersey material, as already mentioned, can be homogeneous in respect of factors influencing butter-fat percentage.

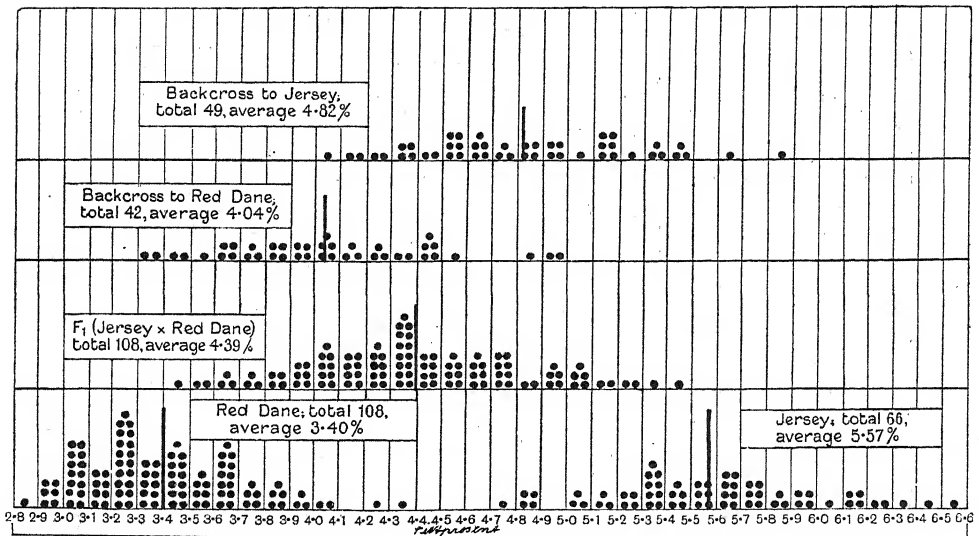


Fig. 13. Chart of the entire cross-breeding analysis.

The hypothesis of one primary factor derives considerable support from the data derived from the two types of back-cross. In the back-crosses with the Jersey the lowest butter-fat percentage found is 4.0, with the Red Danish the highest is 5.0. Between 4.0 and 5.0 we find 22 of the 43 back-crosses with the Red Danish, and 32 of the 49 back-crosses with the Jersey. The fact that both types of back-crosses show so similar a distribution renders any hypothesis of polymeric factors highly improbable. I believe, then, that the best conclusion to be drawn from the material at hand is that the difference in butter-fat percentage

in the milk of Jersey and Red Danish cows is due to a single Mendelian factor. This factor varies in its effect because of the difference in living conditions, and because we are probably dealing with non-genetic variations whose cause is unknown. The conditions are further complicated by the fact that both in Jersey and Red Danish cattle a non-heterogeneous factor composition is found which affects the butter-fat percentage in milk. These factors are not analysed, and therefore the picture is not always clear.

THE GENOTYPE FOR BUTTER-FAT PERCENTAGE IN THE TWO BULLS
MOST USED IN F_1 .

As an example of the way in which these conditions act, I wish to include here a progeny investigation of the two bulls most used in F_1 , of Red Danish and Jersey crossing. One of them, Tystofte, was bred by a Jersey bull and a Red Danish cow with the following butter-fat percentage, 4.10 (4 years' average) and the other, Plus, was bred by a Red Danish bull and a Jersey cow with butter-fat percentage 5.41 (7 years' average). These bulls were mated with very different cross-bred cows. Their ability to inherit the factor for high butter-fat percentage was found to vary greatly. Tystofte produced 31 daughters, of which only one had a butter-fat percentage below 4.0, namely, 3.96. Tystofte was, as a rule, mated with cows with a high butter-fat percentage, the average of the mothers of its daughters was 4.67. In spite of this the daughters had an average butter-fat content in their milk of 4.96.

Fig. 14 shows that the four daughters with a considerably lower percentage of butter-fat than their mothers were all born of mothers with a butter-fat content in their milk above 5.0. Tystofte's genotype for butter-fat percentage is interesting from several points of view. In the first place it shows that he received no heritable factor from his mother for a lower butter-fat percentage than about 4.0. It also substantiates what we have already observed, that the specific Jersey factor for high percentage butter-fat content in milk has the same power to raise the butter-fat percentage in crosses whether it meets a factor for *high* or for *low* butter-fat percentage.

The result of an analysis of the butter-fat percentage of the daughters of the bull Plus, is quite different. There were 35 daughters with a butter-fat percentage averaging 4.35. The butter-fat percentage of the mothers averaged 4.26. Eight of the daughters had a butter-fat percentage below 4.0, and 1 as low as 3.46. On the other hand are 3 individuals segregated with a butter-fat percentage above 5.0. The segregation among the

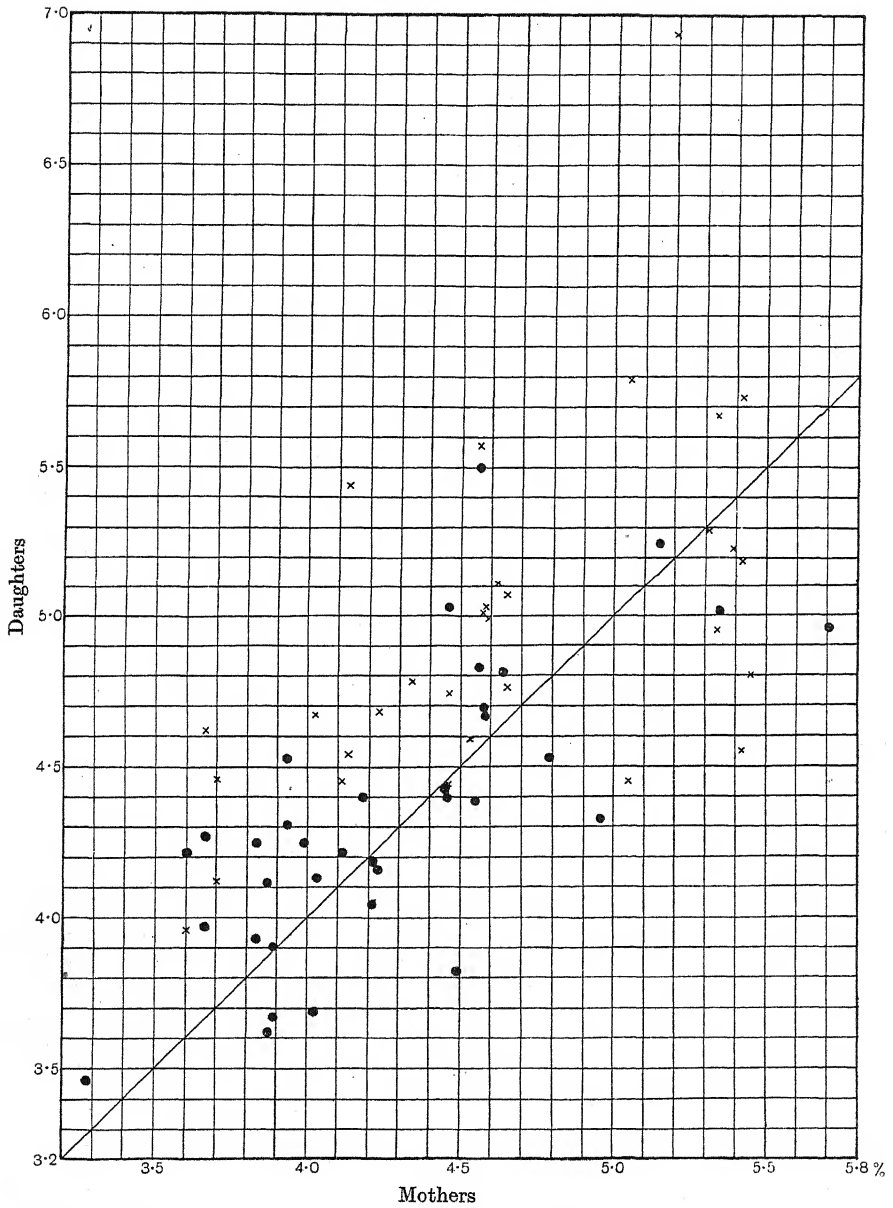


Fig. 14. Graph showing a comparison between the butter-fat percentage in milk of the daughters of Tystofte and Plus as compared with their mothers. Daughters of Tystofte = x ; daughters of Plus = •.

daughters of Plus is just what we should expect in an F_1 bull, from the results of F_1 and crossing back to both parental breeds, in that they vary from 3.4 to 5.5 per cent.

By Tystofte and Plus and one other F_1 bull, who only produced a single offspring, 19 F_2 daughter cows were bred. These illustrate extremely well how valueless the F_2 method is in cross-breeding analyses of this nature, especially when we compare the F_2 cows with their mothers, as appears in Fig. 15. It is worthy of note that there is no difference in variability between F_2 and F_1 .

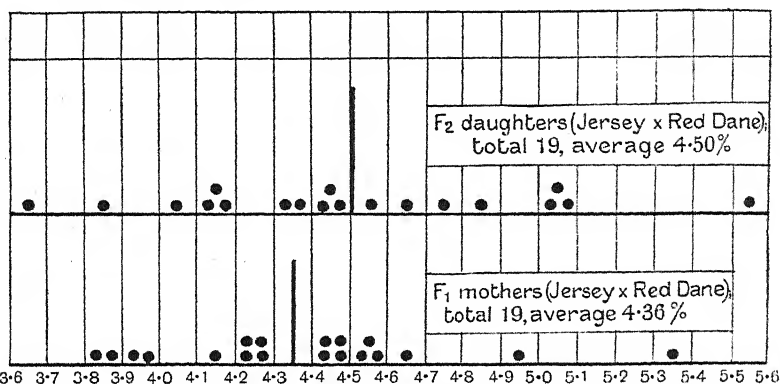


Fig. 15. Graph showing the butter-fat percentage in milk of F_2 cows and in that of their mothers.

On the whole no conclusion can be drawn on the basis of the F_2 data given. Besides the cows already referred to, the investigation material included a series of progeny from different cross-breeding bulls, but the number of the progeny was too small to warrant making an analysis of that material.

In conclusion I have the pleasure of thanking Prof. Lars Fredriksen, Mr Henriksen and Mr Kirkegaard for their most valuable help in these investigations.

CONCLUSION.

As a result of the investigations I state as conclusion that all data seem to indicate that *one* genetic factor exists which causes the difference in content of butter-fat in the milk of the Jersey and Red Danish breeds. This genetic factor varies in its effect, but the effect is equally great whether it meets a gene for high or low percentage butter-fat. Besides this, it is possible that some of the Jersey bulls used in the experiment had a modifying factor which caused an increase in butter-fat percentage, but the existence of this factor has not yet been definitely proved.

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STUDIES IN *PRUNUS*, III.

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(With Thirty-two Text-figures.)

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I. INTRODUCTION.

THE work of Okabe (1927, 1928), Kobel (1927), Meurman (1929 *b*) and myself (1926, 1927, 1928) shows the principles of nuclear differentiation in the genus *Prunus*¹. Its basis is a constant complement of eight chromosomes of distinct form, and the occurrence of polyploidy in all sections of the genus. Beyond this it is interesting that triploids were found amongst cherries and apricots, probably all ornamental forms—for triploidy usually means sterility (Okabe, 1927; Darlington, 1928). Their occurrence indicates the formation of diploid gametes by the diploid parental species of Japanese cherries and hybridisation between the diploid and tetraploid parental species of European cherries. In all these forms a proportion of the odd chromosomes went unpaired, the rest formed trivalents with their homologues. The occurrence of aneuploid varieties of *P. avium* is further evidence of this hybridisation. The behaviour shown in the tetraploid cherries was unexampled amongst natural polyploids, and gave the clue to some problems of genetical behaviour in organisms of this type.

¹ Apart from these Knowlton, Ewert and Dorsey (cf. Darlington, 1928) have examined the chromosome complement in *Prunus*. More recently Asami (1927) has found eight to be the haploid number in the peach.

Two apparent discrepancies should be referred to. First, Okabe (1927, 1928) finds *P. domestica* a diploid, while Kobel (1927) and Darlington (1927) find it a hexaploid. Evidently Okabe's form is more closely related to the Japanese Plum, *P. triflora*, than to the Western species, and this discrepancy shows how, in certain cases, a safer diagnostic test is to be found in cytological than in morphological examination. For, amongst other things, the interfertility of organisms is conditioned by their chromosome number.

Secondly, Meurman (1929 b) finds a high polyploid form in *P. lauro-cerasus*, which however no longer falls into the rigid orthoploid series of lower polyploid forms. This is no argument, as Meurman points out, against the efficacy of the physiological differentiation of the eight chromosomes of the complement: it indicates that high polyploids, on account of the reduced importance of the individual chromosome, will be less affected by the unbalance; and this unbalance, we know, will arise the more frequently in such forms on account of the lower chance of sufficient differentiation between homologous chromosomes of different sets to ensure regular pairing—to ensure, that is, the behaviour of a functional diploid.

My present object is to complete the picture of chromosome constitution and behaviour in the genus, given by other workers, by describing chromosome behaviour in the polyploid plums and damsons (*P. domestica* L. and *P. insititia* L.), the formation of polyploid gametes, and the behaviour of the chromosomes in a series of hybrids connecting *P. domestica* with *P. Amygdalus*.

For the methods used reference may be made to the first of these studies.

II. MATERIAL.

The following varieties have been studied:

P. Amygdalus Stokes: ornamental varieties.

P. persica Stokes: vars. "Chinese Flat Peach"; "Earliest of All"; "Darwin"; ornamental form, Kew.

P. triflora Roxb.: var. "Shiro."

P. cerasifera Ehrh.: "Red Myrobalan."

P. domestica Linn. and *P. insititia* Linn.: varieties of, or hybrids between, these two inextricable species "Cambridge Gage"; "Old Greengage"; "Coe's Violet"; "Comte d'Althan," a Washington seedling¹.

¹ Plant received, 24 February, 1913, from U.S. Dept. of Agriculture, labelled *Prunus domestica* × *spinosa* SPI. 32,673. It is an ordinary *domestica* type and is hexaploid.

P. avium Linn.: vars. "Bigarreau Kentish"; "Bigarreau Noir de Schmidt"; "Governor Wood."

P. spinosa Linn.: wild form, Merton.

The following interspecific hybrids of known parentage:

1. *P. domestica* var. "Jefferson" \times *P. cerasifera* var. "Red Myrobalan," raised by Mr M. B. Crane in 1915. Highly infertile. One seedling has been raised by back-crossing on the female parent. The F_1 has 32 chromosomes (Darlington, 1927, 1928), the back-cross to *P. domestica* var. "Jefferson," 40.

2. *P. triflora* var. "Shiro" \times *P. cerasifera* var. *Pissardii*, raised by Mr M. B. Crane in 1920. It has not set seed. It is apparently heterozygous for the anthocyanin factor of its male parent.

3. *P. triflora* ("Japanese Plum") \times *P. persica* var. "Sea Eagle," raised by Mr W. Laxton (cf. Laxton, 1906, for illustration). Completely sterile, usually having the pistil malformed. The petals have the light pink colour of the male parent.

4. *P. persica* variety \times *P. Amygdalus* variety of bitter almond; raised by Messrs Laxton. The fruit is fleshy but flavourless. Only eight seedlings were raised from large numbers of flowers selfed in the house (1911, 1912). These seedlings lack vigour, and have never yet set seed themselves. None, therefore, has the appearance of being tetraploid.

I am indebted for seeds of *P. Fenzliana* to Prof. Sosnowsky, Director of the Tiflis Botanical Garden, and for flower buds of *P. lannesiana amabilis* and other forms to the Director of the Royal Botanic Gardens, Kew.

III. POLLEN MOTHER-CELL DIVISIONS.

(1) General.

The chromosome forms in somatic divisions and in meiosis are indistinguishable in the various sections of *Prunus*. A somatic division in *P. Fenzliana* with the diploid number of 16 illustrates chromosome form at mitosis (Fig. 1), and a side view of a pollen mother-cell division in *P. lannesiana amabilis* ($n = 8$) illustrates the characteristic structure of the bivalent chromosomes at meiosis (Fig. 12 B). If we are to compare these structures with those of larger chromosomes at the same stage we must allow, first, for their extremely small size which obscures the finer details, and, secondly, for the tension which draws out into a fine thread the portion of each chromatid (half chromosome) between its point of "attachment" to the spindle and the chiasma where it changes its asso-

ciation becoming attached to a fellow chromatid attached to the opposite pole. This effect is not usually observed in the largest chromosomes¹, but is found in certain chromosomes in *Tulipa Clusiana* and *Fritillaria Meleagris* (Newton and Darlington, 1929, Fig. 15; 1930, Fig. 7). Examination of every stage of separation shows very clearly that the structure of the bivalent chromosome in *Prunus* is of a simple and well-recognised



Fig. 1. Mitosis in the root-tip of *Prunus Fenzliana* ($2n=16$). ($\times 4100$.) Note: the chromosomes are 1 to 2.5μ long. In *Hyacinthus orientalis* the longest chromosome is 21μ long.

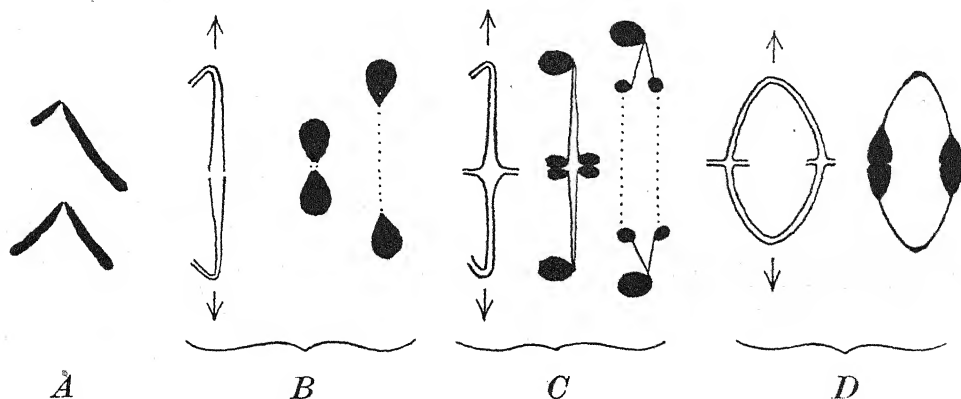


Fig. 2. Diagram showing the chiasma structure of bivalent chromosomes at metaphase and anaphase in *Prunus* species. (Cf. Fig. 12.) A. Types of somatic chromosomes. B. Type of bivalent with terminal chiasma, first representing the relations of the chromatids, second and third, the actual appearance of bivalents at metaphase and anaphase. C. The same with sub-terminal or median chiasma. D. The same with two sub-terminal chiasmata. Only occurs probably with the long medianly constricted chromosome type. Anaphase not observed. ($\times ca. 10,000$)

type. It consists of four chromatids associated in pairs but with a change of partners at one or possibly two, usually interstitial, points, the "chiasmata" (cf. diagram, Fig. 2).

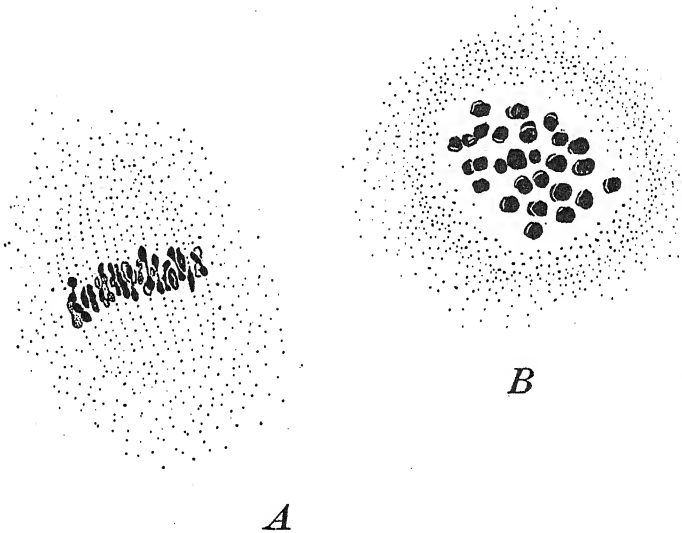
The accounts of Kobel (1927) and Okabe (1928) make unnecessary any further description of the diploid species *P. cerasifera*, *P. triflora*,

¹ This might be due to a weakness of the chromosome characteristic of certain points, and analogous to a constriction. It might also be due to the first chiasma being exceptionally close to the attachment constriction.

P. persica and *P. Amygdalus*. It was in varieties of *P. persica* and *P. Amygdalus* that the greatest irregularities of pairing were observed (cf. section 4).

(2) *Hexaploid and tetraploid species.*

All the forms of plums and damsons examined were hexaploid. As a rule 24 bivalents are formed (Fig. 3 *A*), as recorded by Kobel (1927). The exceptional cases are, however, important. Secondary pairing occurs, and this, as I have suggested (cf. Meurman, 1929 *b*), is probably nothing but quadrivalent formation where the connection between the chromosomes is too tenuous to be seen. In certain cases the quadrivalent nature



Figs. 3-7. *P. domestica*. First metaphase. ($\times 2400$)

Fig. 3. Washington seedling plum: *A*. 24 bivalents. *B*. 22 bivalents, 1 quadrivalent (centre); in this plate the chromosomes are swollen to an extent not observed in the best fixations.

is unmistakable as where the figure is diamond-shaped (centre of Fig. 3 *B*). The formation of trivalents and univalents, as in the cherries, is associated with that of quadrivalents (Figs. 4-7). Irregularity of segregation necessarily follows this irregularity of pairing. As many as eight univalents have been observed lagging to divide on the equator, while the paired chromosomes separate to the poles (Fig. 8). In one case the division of a univalent side by side with that of a probable trivalent was observed at anaphase (Fig. 9).

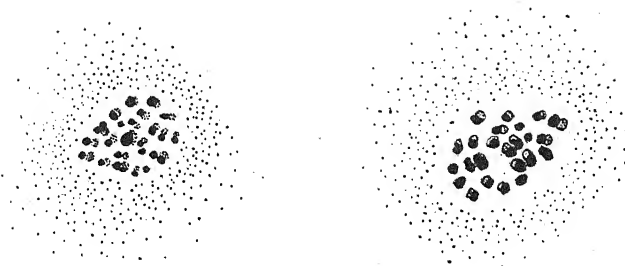


Fig. 4. Coe's Violet (early anaphase): 18 bivalents, 3 trivalents, 3 univalents.

Fig. 5. Cambridge Gage: 8 bivalents, 3 secondary pairs (quadrivalents), 2 probable trivalents, 2 univalents.

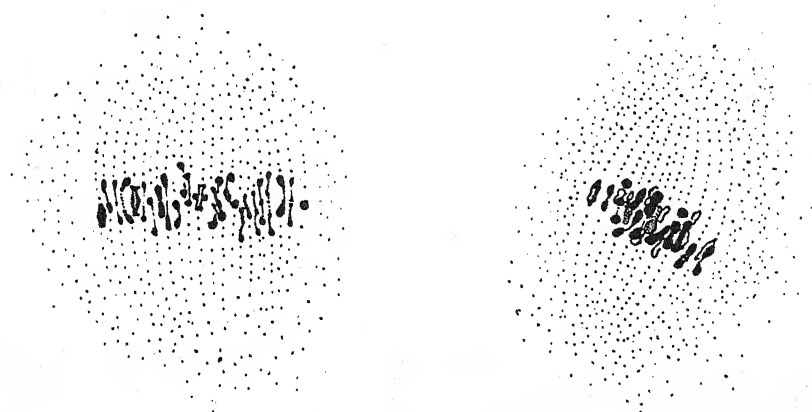


Fig. 6. Coe's Violet: 22 bivalents, 1 trivalent, 1 univalent (chromosomes drawn separately).

Fig. 7. Washington Seedling: 2 trivalents.

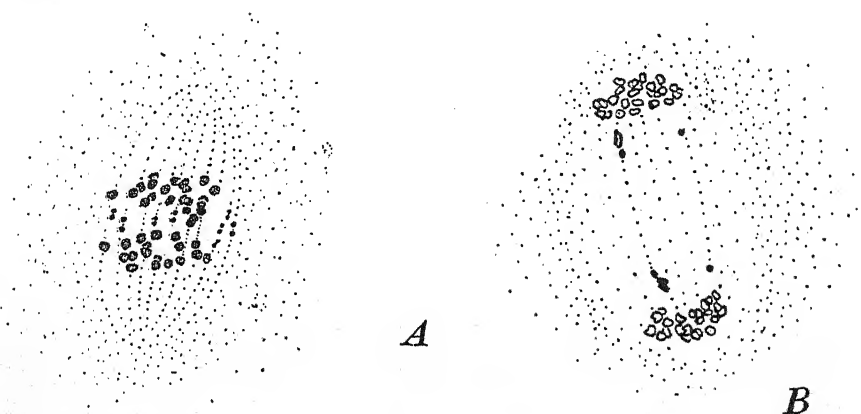


Fig. 8. First anaphase in *P. domestica* var. Old Greengage: A. 8 univalents dividing after 20 chromosomes have separated to each pole. B. A univalent and a probable trivalent lagging after dividing. ($\times 2400$)

At the second division laggards are consequently found lying in the cytoplasm, and irregular numbers of chromosomes make up the plates (Fig. 10).

In a wild seedling of *P. spinosa*, side by side with perfect pairing, two abnormalities were found: first failure of pairing (Fig. 11); secondly

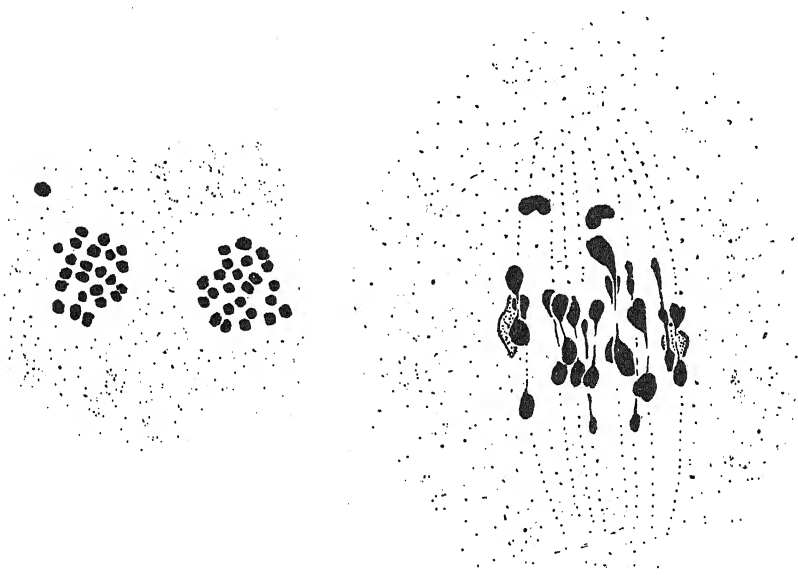


Fig. 9. Second metaphase in *P. domestica* var. Comte d'Althan (supposed hybrid with *P. insititia*): $24+23+1$. Secondary pairing persists. ($\times 3500$)

Fig. 10. *Prunus spinosa*. First metaphase: 15 bivalents, 2 univalents. ($\times 5000$)

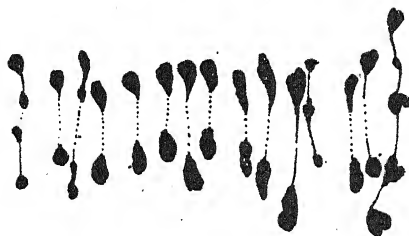


Fig. 11. *P. spinosa*. First metaphase. Chromosomes drawn separately. 14 bivalents, 1 quadrivalent. ($\times 5000$)

the formation of one or two quadrivalents (Fig. 12) of various forms. Although I do not attach much importance to the distinction between wild and cultivated forms of *Prunus*, it will be of interest to some to notice these peculiarities in an authentic and fairly homogeneous seed-

producing species. Quadrivalents however are less frequent than in most varieties of *P. cerasus* that have been studied.

(3) *The hybrid chain; autosynopsis.*

The series of hybrids between the species, *P. domestica*—*P. cerasifera*—*P. triflora*—*P. persica*—*P. Amygdalus*, have been examined. Although irregularities occur, perfect pairing is found, in each of the hybrids, between homologous chromosomes of different species (Figs. 11 B-G, 12 and 15). This I take to be a demonstration that, as organs, the eight chromosomes of *P. domestica* and *P. Amygdalus* are homologous each to each notwithstanding the changes that may have taken place in the period separating them from their common origin. The pairing proves what had previously, as is usual in these cases, been inferred from comparison of chromosome form and number in each species. This historical correspondence may very well conceal a complete lack of material correspondence at opposite ends of the chain, owing to interchanges of material in the course of evolution, which would thus result in as complete a failure of pairing between the chromosomes of the species at the opposite ends of the series as there is, for example, between the probably homologous nine chromosomes of *Raphanus* and *Brassica* (Karpechenko, 1927).

Failure of pairing is particularly frequent in the peach-almond hybrid, with resultant irregularity (Fig. 14; see also later).

As mentioned earlier (1927) the behaviour of the hybrid *P. domestica* × *P. cerasifera* would be characteristic for a tetraploid *Prunus* species such as *P. cerasus*. The irregularities that occur are due to the homologous chromosomes having too great an affinity for one another, rather than too little. Secondary pairing is very marked, and persists to the second division (Figs. 18 and 19). This persistence earlier seemed to distinguish the secondary pairing from the ordinary formation of quadrivalents, which I had otherwise observed only in Monocotyledons with a marked interphase. It is, however, possible to suppose that chiasmata that have not unravelled at the first division, owing to the associated chromosomes passing to the same pole, may persist to the second division, in the absence of any interphase, although they might disappear during an interphase.

As in the other polyploid forms trivalents and univalents may take the place of quadrivalents at metaphase with consequent irregularity of segregation (Figs. 16, 18–20).

The tetraploid behaviour of the hexaploid-diploid hybrid must be due

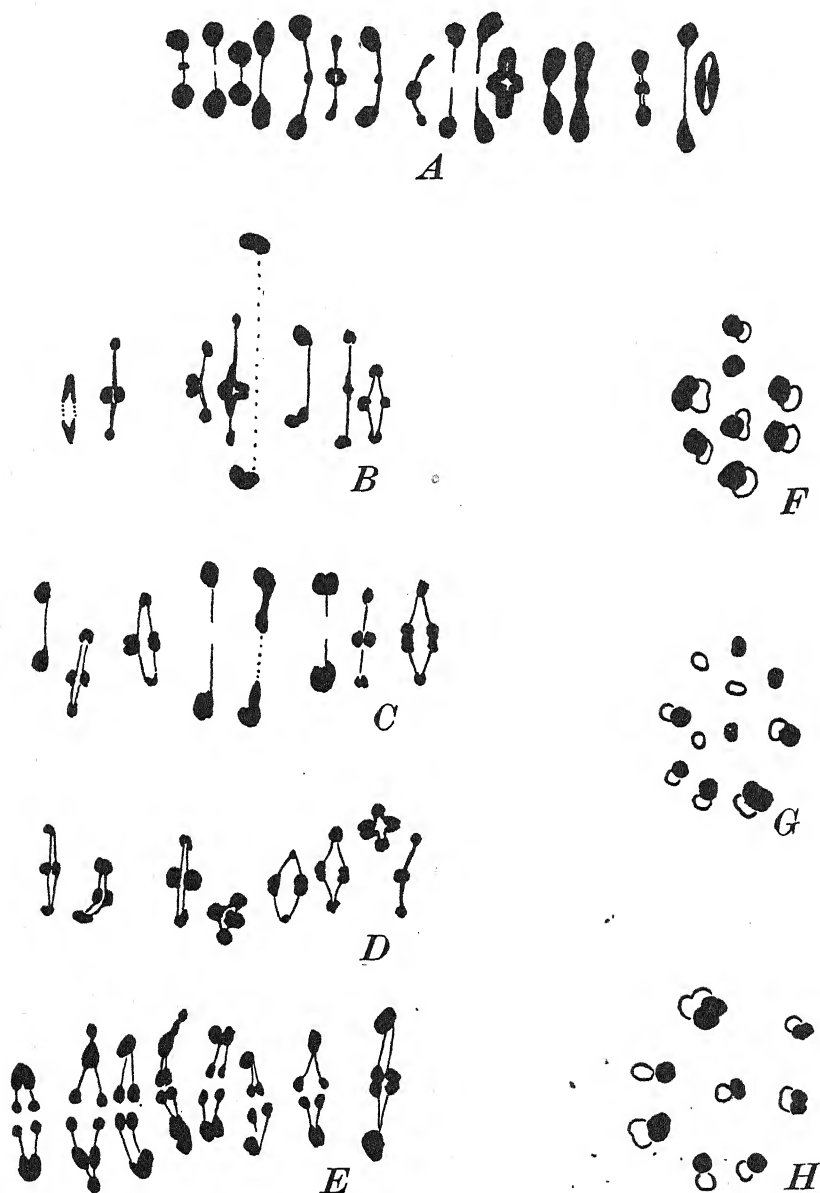


Fig. 12. *A* and *C-H*. The hybrid chain in *Prunus*: *A*. *P. domestica* \times *P. cerasifera* ($n=16$); *C* and *F*. *P. triflora* \times *P. cerasifera* ($n=8$); *D* and *G*. *P. triflora* \times *P. persica* ($n=8$); *E* and *H*. *P. persica* \times *P. Amygdalus* ($n=8$); *E*. Early anaphase. The rest, first metaphase. *B*. *P. lannesiana amabilis* ($n=8$). ($\times 5700$.) Note: bivalents with terminal chiasmata separate first.

to the pairing of the set from the diploid with one of the three sets from the hexaploid, while the other two sets of the latter pair together: autosyndesis is complete (cf. Haase-Bessel, 1922¹; Collins and Mann, 1923; Ljungdahl, 1924; Crane and Darlington, 1928, and others). This



Fig. 13. Diakinesis in *P. triflora* × *P. persica* ($n=8$). ($\times 2800$)

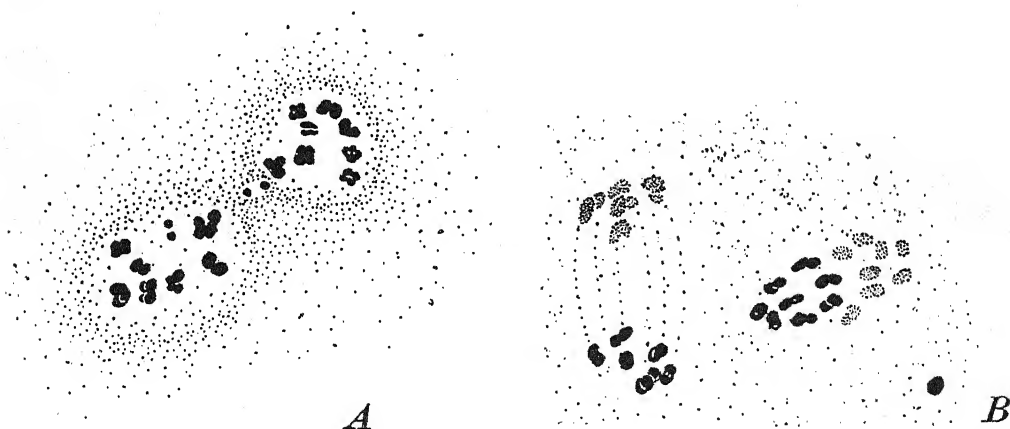
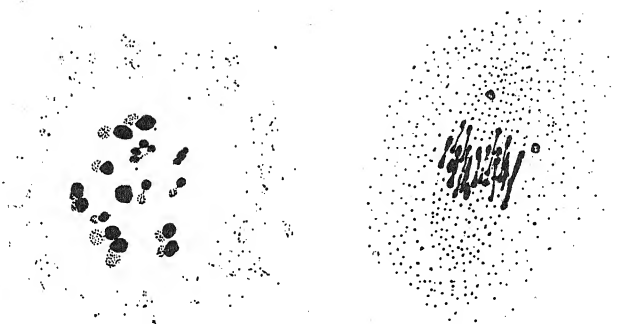


Fig. 14. *P. persica* × *P. Amygdalus*. A. Second metaphase (early): $8 + \frac{1}{2} + 7\frac{1}{2}$. Showing how a lagging chromosome may form a bridge between nuclei. B. Second anaphase: $8 + 8, 8 + 7 + 1$. ($\times 4100$)

analysis of *P. domestica* is a demonstration of the correctness of the assumption of polyploidy in the same way as is the artificial synthesis of a polyploid. Its genetical implications have been considered in detail (Darlington, 1928).

¹ Buxton and Newton (1928) find that the chromosome numbers are multiples of 14, not 12 as Haase-Bessel states, and I have been able to confirm their observations.



Figs. 15-20. *P. domestica* \times *P. cerasifera*.

Fig. 15. First metaphase: 5 quadrivalents and 6 bivalents. ($\times 4300$)

Fig. 16. First metaphase: 13 bivalents, 1 trivalent, 3 univalents. ($\times 2800$)

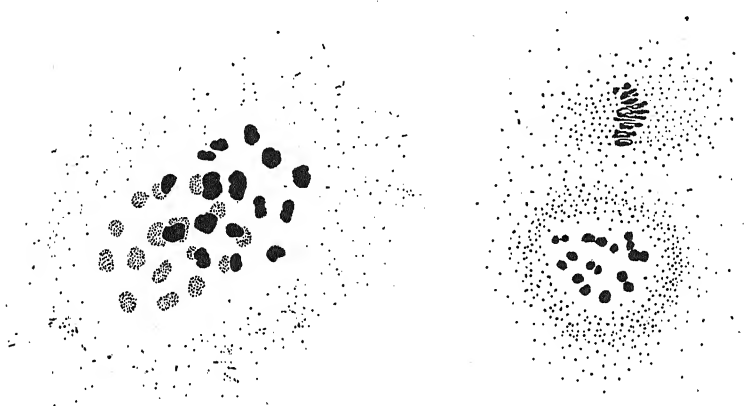


Fig. 17. First anaphase: regular. Secondary pairing. ($\times 4100$)

Fig. 18. Second metaphase. Secondary pairing and two divided univalents in one plate. ($\times 2800$)

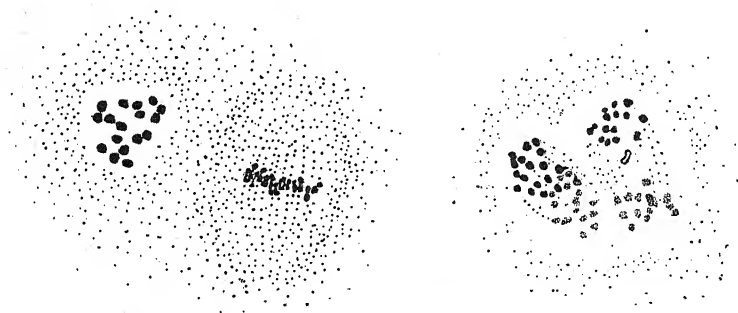


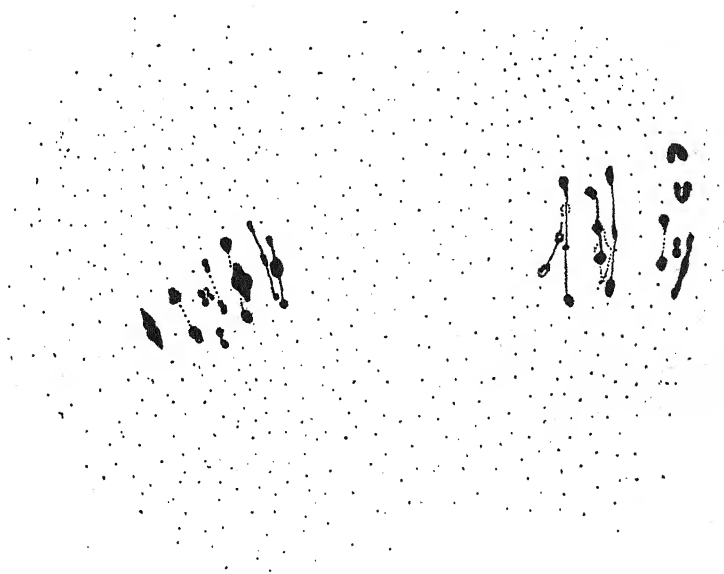
Fig. 19. Second metaphase: 15 + 17. Secondary pairing. ($\times 2800$)

Fig. 20. Second anaphase: 17 + 17, 16 + 14. ($\times 2800$)

(4) *Formation of polyploid gametes.*

Two important irregularities of spore formation have been found which would lead to the production of gametes with an unreduced or even increased number of chromosomes.

In the three varieties of Sweet Cherry (*P. avium*) binuclear pollen mother-cells are occasionally found. In meiosis two nuclei may divide side by side (Fig. 21), or their spindles may unite so that, for the moment, a tetraploid group is formed. It is apparently characteristic that this union should be imperfect (Fig. 22) for Kagawa (1928, Fig. 33) illustrates the same peculiarity. In the first case the fusion will take place, if at



Figs. 21-23. *P. avium* var. Governor Wood.

Fig. 21. Incomplete syndiploidy at first metaphase. ($\times 4100$)

all, at the second division, and the results will be irregular. The dyads and monads observed (Fig. 23) probably result from this imperfect fusion at the first division, and their nuclei will have a tetraploid or higher number of chromosomes.

The second kind of irregularity was observed most frequently in the peach-almond hybrid, but evidence of it was found also in the "Chinese Flat Peach" in *P. Amygdalus* and in the *P. triflora-persica* hybrid. Here single nuclei are formed at the end of the first division, owing to the two

separated groups of chromosomes being connected by laggards (Figs. 24 and 25). These "restitution nuclei" (cf. Rosenberg, 1926) divide once to

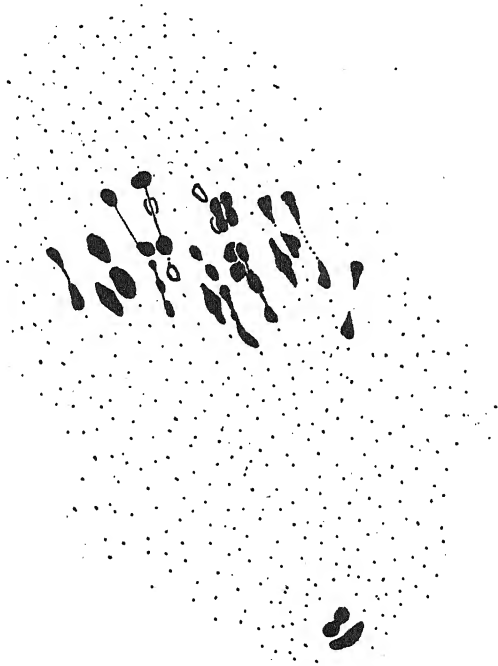


Fig. 22. Syndiploidy nearly complete; two bivalents left out. ($\times 4100$)

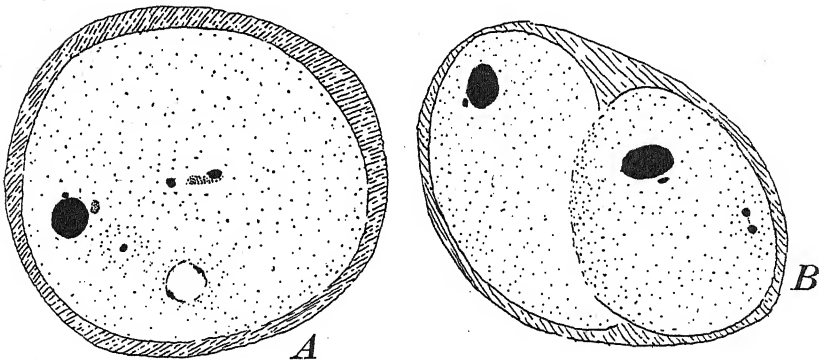
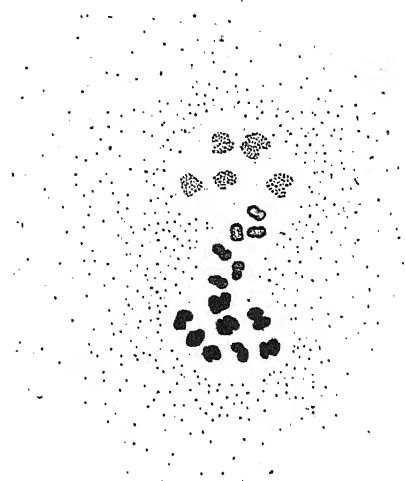


Fig. 23. A. A multinuclear monad. B. A dyad (from a smear preparation). ($\times 2400$)

give a dyad (Fig. 27) each nucleus having the diploid number of chromosomes. At this second division the homologous pairs of chromosomes



Figs. 24-27. *P. persica* \times *P. Amygdalus*.

Fig. 24. Second metaphase following the lagging of three divided univalents and the formation of an imperfect restitution nucleus. ($\times 4100$)

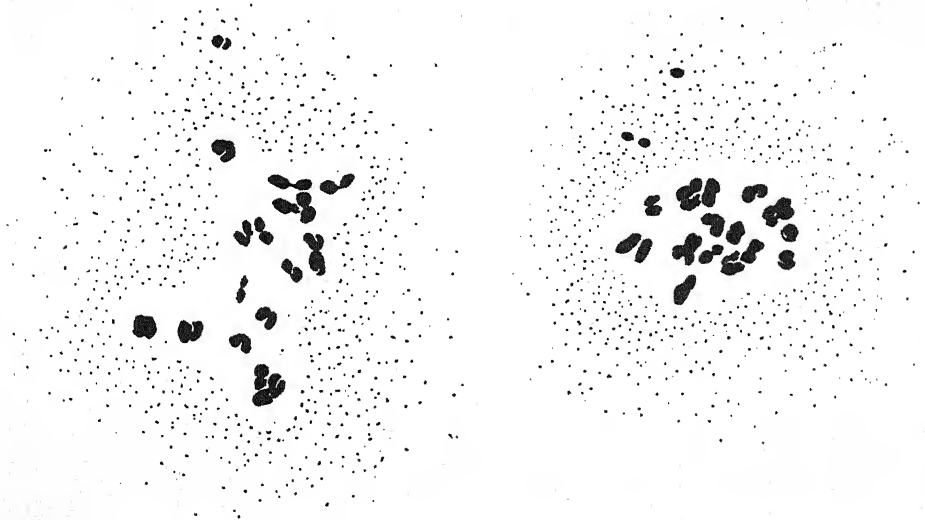


Fig. 25. Second metaphases from imperfect restitution nuclei. ($\times 4100$)

may continue to show the connections of the first division, thus giving an appearance of a second division in a tetraploid with secondary pairing (Fig. 26). The irregularities were concentrated in certain anthers here and in *P. avium nana*.

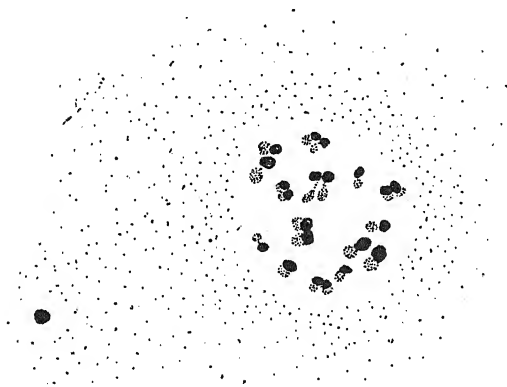


Fig. 26. Second metaphase: $15 + 1$. The approximately diploid plate shows secondary pairing of the corresponding chromosomes. Adjoining division shown in Fig. 14 B. ($\times 4100$)

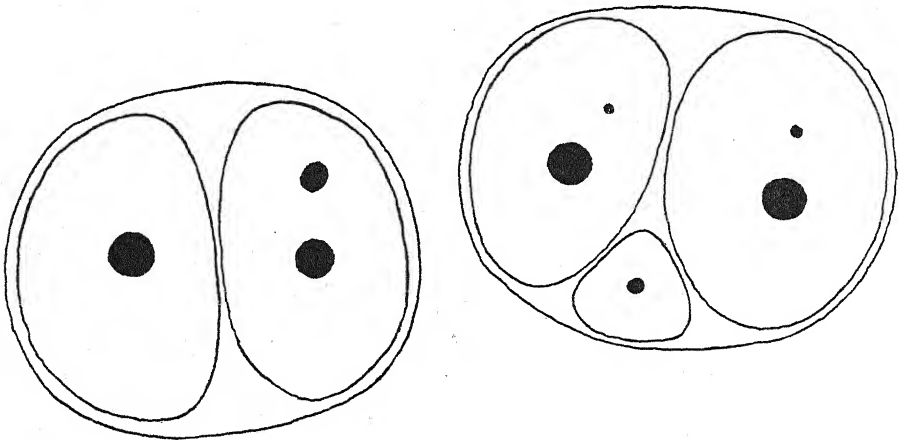
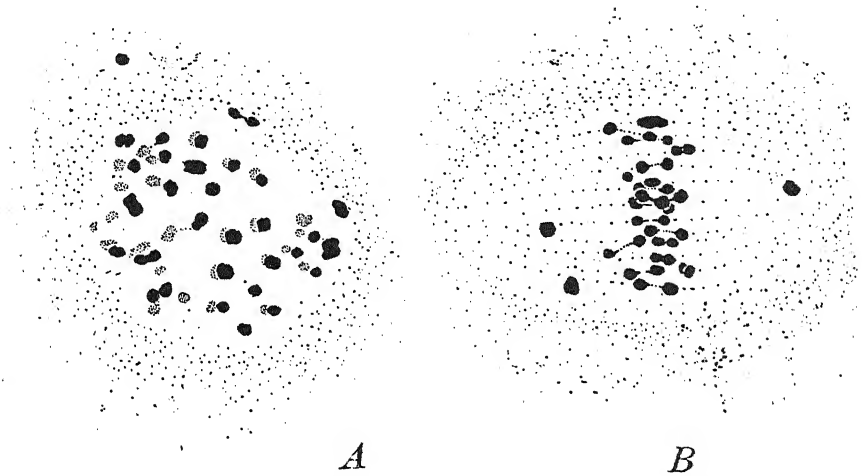


Fig. 27. Dyads formed from restitution nuclei (side by side in a smear). ($\times 2400$)

In the triploid cherry, *P. avium nana*, both these abnormalities evidently occur, although all the stages of each have not been found. Where a binuclear pollen mother-cell has divided we have two second division plates each with the triploid number (Fig. 28). Where a restitution nucleus has been formed a single second division is found with the

triploid number. In the case illustrated (Fig. 29), in each anaphase group the number is greater than 24. This suggests that some of the chromo-



Figs. 28-32. Irregular pollen mother-cell divisions in the triploid *P. avium nana*. ($\times 4100$)

Fig. 28. Successive sections showing the two second divisions in one syndiploid cell.
A. Interphase. B. Metaphase.

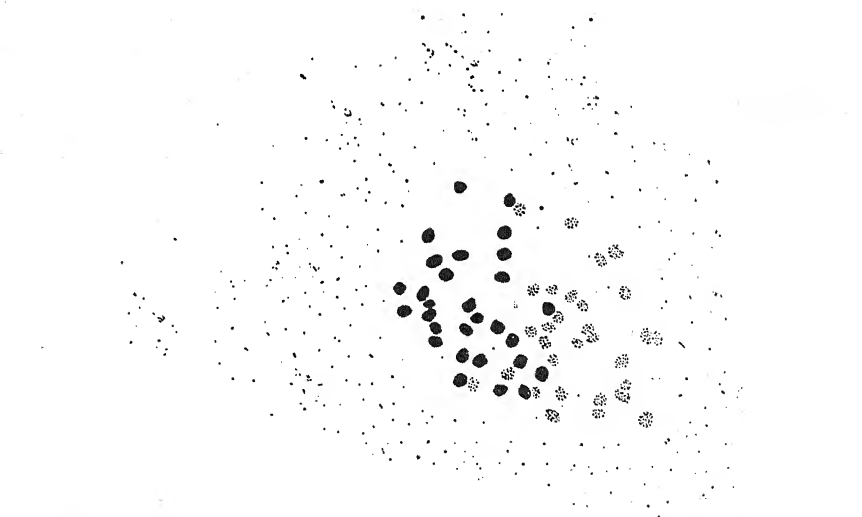


Fig. 29. Second anaphase after formation of a restitution nucleus. There are more than 24 chromosomes in each group, indicating that some univalents have divided twice.

somes have divided twice, a third abnormality. We may get imperfect formation of a restitution nucleus (Fig. 30), and we may get the

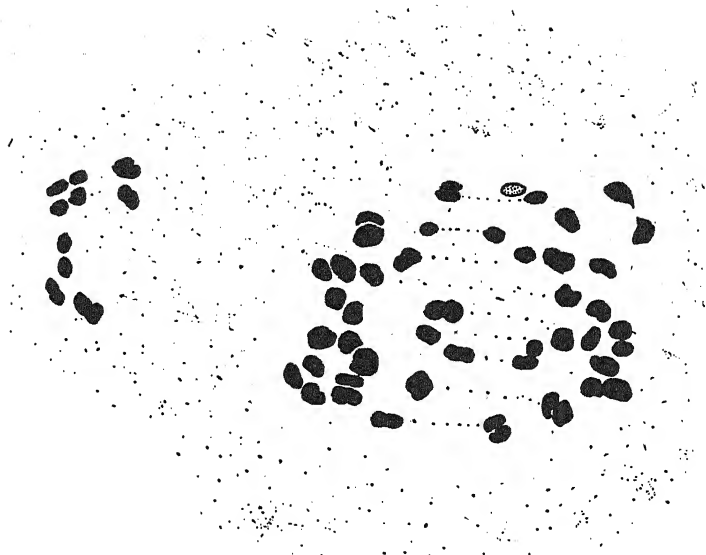


Fig. 30. First anaphase after incomplete syndiploidy. Some univalents dividing

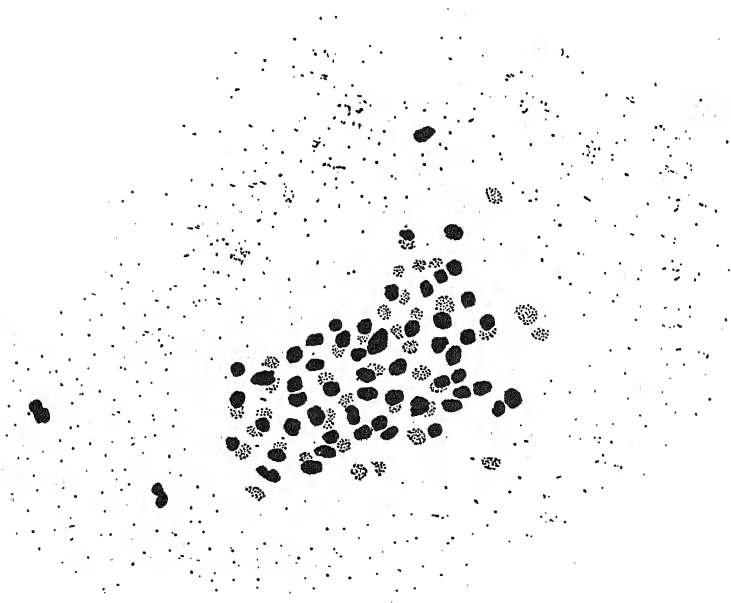


Fig. 31. Formation of a secondary restitution nucleus (both divisions abortive) in a syndiploid cell. About 96 chromosomes present.

combination of all these irregularities where, owing to the failure of any nuclear division, the double and divided complement is contained in one nucleus (Fig. 31) with about 96 chromosomes. It would appear also that failure of the second division may occur following a perfect first division (Fig. 32).

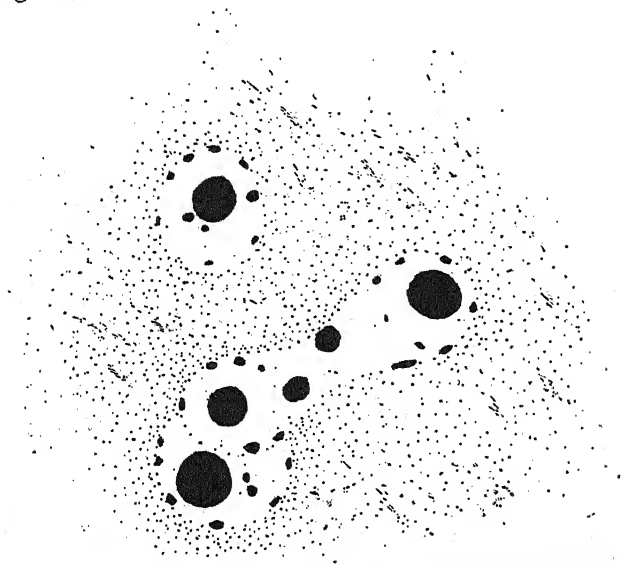


Fig. 32. Formation of a secondary restitution nucleus by the abortion of the second division.

IV. CONCLUSIONS.

(1) *The origin of polyploids.*

We may conveniently arrange abnormalities of the germ-cell maturation divisions leading to the formation of polyploid gametes as follows: A. The nullification of the first or second division. B. The double division of the chromosomes. C. Syndiploidy or the formation of binuclear germ mother-cells. D. The complete suppression of the first division. Instances in these groups may be distinguished by the conditions of their origin and by their genetical effects. The following is a list of earlier observations all of which, except the last, have been repeated in *Prunus*¹.

¹ Compare also Brieger (1928). The doubling in the embryo-sac of some species of *Lilium* and *Tulipa* (cf. Newton, 1927) is strictly a somatic irregularity. The observation of a failure of numerical reduction in certain *Caninae* roses (Täckholm, 1922) is more properly classed as an abnormal type of segregation. Tetraploid germ mother-cells in *Drosophila* (Morgan, Bridges and Sturtevant, 1925) and in *Oenothera* (Håkansson, 1927) arose probably from doubling some time before meiosis (somatically).

A.

- Juel, *Taraxacum* ♀, 1904, 1905
 Meves¹, *Apis* (n), 1907
 Osawa, *Taraxacum*, 1913
 Rosenberg, *Hieracium* (2n, 3n), 1917, 1926
 Sakamura, *Vicia*, 1920*
 Yasui, *Papaver*, 1921
 Blakeslee, Belling, Farnham and Bergner, *Datura* (n), 1922. Belling, 1928
 Belling and Blakeslee, *Datura* (3n), 1922
 Ljungdahl, *Papaver*, 1922
 Bremer, *Saccharum*, 1923
 Kihara, *Triticum-Secale*, 1924
 v. Wettstein, *Funaria*, 1924*
 Michaelis, *Epilobium*, 1926*
 Sakamura and Stow, *Gagea*, 1926*
 Florin, *Syringa*, 1926
 Gaines and Aase, *Triticum* (n), 1926
 Randolph and McClintock, *Zea*, 1926
 Matsuda, *Petunia*, 1927
 Karpechenko, *Raphanus-Brassica*, 1927
 Rybin, *Nicotiana*, 1927
 Kobel, *Prunus*², 1927
 Shimotomai, *Liriope*, *Scilla*, 1927*
 Tischler, *Ribes*, 1927
 Schwemmle, *Oenothera* (4n), 1928
 Kuwada, *Balanophora* ♀, 1928
 Brieger, *Nicotiana* (3n and 5n), 1928
 Huskins, *Avena* (6n-2), 1928
 Buxton and Newton, *Digitalis*, 1928
 Lesley and Frost, *Matthiola* (n), 1928

- Takagi, *Lychnis*, 1928*
 Kagawa, *Triticum-Aegilops*, 1929
 Meurman, *Aucuba*, 1929 a
 Newton and Darlington, *Tulipa* (3n), 1929
 Darlington, *Tradescantia* (3n), 1929 b
 Håkansson, *Salix* (3n), 1929
 Davis and Kulkarni, *Oenothera* (n), 1930
 * Effects induced experimentally

B.

- Federley, *Pygaera*, 1913
 Clausen³, *Viola*, 1926
 Karpechenko, *Raphanus-Brassica*, 1927
 Meurman, *Ribes*, 1928

C.

- Geerts, *Oenothera*, 1909
 Gates and Rees, *Lactuca*, 1921
 Randolph and McClintock, *Zea*, 1926
 Florin, *Syringa*, 1926
 Gaines and Aase, *Triticum* (n), 1926
 Håkansson, *Oenothera*⁴, 1927
 Kobel, *Malus*, 1927
 Karpechenko, *Raphanus-Brassica*, 1927-8
 Kagawa, *Triticum-Aegilops*, 1928-9
 Nebel, *Malus*, 1929

D.

- Juel, *Antennaria* ♀, 1900
 Rosenberg, *Hieracium*, 1917, 1926
 Lesley and Frost, (?) *Matthiola* (n), 1928

The immediate causes of these various types of irregularity are fairly clear in *Prunus*, and appear to operate in most other cases. The first type occurs (naturally) only where chromosomes lag in the first division, as a result of failure of pairing or, in the second, as a result of having already divided in the first; thus the formation of two separate nuclei

¹ Cf. Bělař, *Die Cytologischen Grundlagen der Vererbung*, 1928.

² Kobel's observation on *Prunus domestica* probably falls in this class. His observation of complete failure of pairing at diakinesis in *P. cerasifera* also suggests rather a restitution nucleus resulting from imperfect pairing.

³ Clausen suggests that "the chromosomes, already once divided, seem to divide again," but the increase of chromosome number in the progeny of this *Viola* hybrid might be due to the formation of restitution nuclei.

⁴ Probably doubled three divisions before meiosis.

is prevented¹. It appears to occur as freely in plants with prolonged interphase as in those with the shortest interval between the first and the second divisions. It is not a symptom of hybridity as such², for it arises particularly in haploids and in triploids which are not hybrids (e.g. *Tulipa*). It should be noted also that it occurs in plants which are not systematically regarded as hybrids, such as *Nicotiana Tabacum* (Rybin), a species in which failure of pairing is a symptom of auto-polyploidy, that is lack of hybridity. The localisation of irregularities in *Prunus* and probably *Digitalis* is evidence that their occurrence is not always independent of extra-nuclear conditions. External conditions probably play some part in most cases in promoting this irregularity in nucleus formation³, and may even replace the natural cause, failure of pairing, as is shown by the work of Wettstein, Sakamura and Stow and others. Amongst these variations must be numbered the difference between the conditions of male and female spore mother-cells (*Taraxacum*). Single "restitution" nuclei (Rosenberg, 1926) formed as a result of failure of the first division probably need not give rise to diploid dyads: in *Aucuba* multinucleate cells are produced. Time irregularities are commonly observed in connection with the formation of restitution nuclei in *Prunus*. As in *Aucuba*, I have found that abnormal cells are usually retarded, but sometimes, as in *Hieracium* (Rosenberg, 1926), they are advanced (cf. also Rybin, 1927).

The second type of irregularity has been shown most clearly in *Pygaera* and *Ribes* in association with the complete failure of pairing at the first metaphase. In the present case, *P. avium nana*, as in *Raphanus-Brassica*, the determination of a double division of the univalent depends on counting the products of the second division. The fact that this double division may occur where interphase is of the shortest duration points to a complete failure of association at pachytene as the condition of this abnormality for the abnormality must be determined some time before.

The fact that the occurrence of this type of division in *Ribes Culverwellii* and *Ribes Gordonianum* is sporadic (as well as in most of the other

¹ This is probably not always the case in *Aucuba* (Meurman), where restitution nuclei arise, no doubt following the lagging of chromosomes which have been paired in complex configurations (cf. *Hyacinthus*, Darlington, 1929 a).

² Dissimilarity of chromosomes has been shown to be only one of the circumstances in which they may fail to pair (cf. Darlington, 1929 a, b).

³ Rybin attributed the formation of restitution nuclei in *Nicotiana* to a fall in temperature, and Buxton and Newton attributed the special distribution of fertile seeds in their hybrid to the effect of external influences.

examples) is not incompatible with this. For we may regard the failure of pairing (at pachytene and hence at metaphase) as due to the occurrence of structural differences between the corresponding chromosomes. Thus, if the materials that make up two chromosomes correspond, but their arrangement is different, a particular correspondence continuing only for a very short length along the pair, then the completion of pairing must be very difficult mechanically, and the chromosomes will suffer from the same disadvantage and the same randomness in pairing as do small fragments (Darlington, 1929*b*).

The third type of irregularity, "syndiploidy," occurs only in *P. avium*. It appears rather to be a genetic property of certain varieties of this species, and has no special connection with hybridity or failure of pairing in spite of its occurring in *Raphanus-Brassica*. I regard it rather as the first stage of contabescence in an anther, related perhaps therefore to male sterility, which occurs in *P. persica* and *P. domestica* (Crane and Lawrence, 1929) and in *P. cerasus*, and is a unit character in *Rubus* and elsewhere (Crane, 1926).

The fourth type of irregularity, the suppression of the first division, occurs side by side with the first and has the same morphological consequence, the formation of dyads (although, were these functional, the genetic consequences might be different). For these reasons Rosenberg concludes that there is "*kein prinzipieller Unterschied*" between the two types. But a theoretical distinction can be made which is perhaps not unimportant. In the first type the failure of separation can often be attributed to lagging (from various causes) of chromosomes which then form a bridge between the two daughter nuclei. In the fourth type no such crude mechanism can be pointed to as the cause.

Lack of pairing amongst the chromosomes, although it never interferes with the contraction of the chromosomes characteristic of meiosis¹, is apparently a condition equally of this suppression and of double division (B), and it is interesting for the theory of meiosis to note that such different consequents can follow the same antecedent. In one case the habit of single division of the chromosome is maintained at the expense of the habit of double division of the nucleus; in the other the double division of the nucleus is maintained at the expense of the single division of the chromosomes. In this case the mechanism of division is able, as it were, to control the division of the chromosomes, but it need not follow that this mechanism

¹ Where lack of contraction is observed this seems a good ground for regarding the division as a second division following a suppressed first division. This is the case in the haploid *Matthiola*.

is originally independent of chromosome pairing. Fertilisation with a reduplication of chromatin material is an invariable antecedent of normal meiosis. It is easier, therefore, to consider the condition of the chromatin as of fundamental importance and to regard the rapid sequence of the two divisions as determined by the temporary association of pairs of chromosomes between zygotene and diplotene, this association being followed by the indifferent separation of chromatids in pairs (the formation of chiasmata) and the reproduction at *anaphase* of the first division of a condition characteristic of *prophase*. In the absence of this association the stimulus to division is postponed, and only the primitive single division takes place (*Hieracium pseudoillyricum*).

Where we get all possible types of irregularity, as in *Raphanus-Brassica* and *P. avium nana*, germ-cells with very high chromosome numbers can be produced. In *Raphanus-Brassica* such germ-cells have been shown to function, and the existence of a species of *Prunus*, with about 180 chromosomes (Meurman, 1929), shows the same possibility here although such a form need not have arisen directly from diploids and tetraploids.

The effect on segregation of these four types of irregularities will be different (cf. Crane and Darlington, 1928). Wettstein showed that after nullification of the second division in heterozygous *Funaria hygrometrica* homozygous diploid spores were obtained (1924). But, since the first division has often been shown to be equational, this need not always follow. As far as paired chromosomes are concerned, in the case where reduction takes place equally often at the first and second divisions, suppression of the second will give gametes, having the heterozygosity of the products of self-fertilisation (1 : 2 : 1); suppression of the first will give a segregation in heterozygous factors of 1 : 6 : 1. (The increased proportion of heterozygotes in the second case is due to the fact that suppression of the first division allows free recombination between all four chromatids, while suppression of the second does not.) Double division or suppression of the first division without pairing will give no segregation. The gametes will have the full heterozygosity of the parent. Perfect syndiploidy will give the same result as suppression of the second division.

The fact that, in certain cases where segregation would be expected, it has not been observed in experiment is no doubt due to the elimination of segregates in interspecific hybrids, but the observation of sterile individuals by Karpechenko (1927) amongst his tetraploid seedlings is to be attributed to segregation perhaps following crossing-over. Asso-

ciation, and therefore crossing-over, may occur at prophase between chromosomes that are no longer paired at metaphase.

Winge (1917) has suggested that polyploids arise as a *result* of hybridity in their diploid parents. He seeks to explain the origin of polyploids in the following terms:

Where a less-marked harmony exists, we must then suppose that this will be visibly expressed by the fact that the chromosomes derived from the two gametes will not unite in pairs at all, but will distribute themselves through the primary cell of the zygote as if no dualistic relation of any kind existed. If the chromosomes are to find partners, then each of the chromosomes in the zygote must divide, for this indirectly to produce a union of chromosomes, and we must assume that this is realized in the hybrid zygotes which have any possibility at all of propagating—in accordance with what we know from experience of the behaviour of pairs of chromosomes.

According to Winge therefore the necessity for the existence of pairs leads to a doubling at this stage, called "indirect chromosome union¹." This hypothesis has been a useful basis for argument but the phrasing leaves some doubt as to whether it is not for the purpose of pairing that the chromosomes are supposed to double. The ambiguity seems to have created some confusion, for Clausen (1926), observing the production of gametes with an incompletely reduced number of chromosomes in a *Viola* hybrid, concludes that "Winge's hypothesis must therefore be said to have been confirmed by experiment." Jørgensen, however (1927), maintains that not only have the processes imagined by Winge never been found, but that "in all probability they never will be found." This conclusion is probably nearer the mark. Doubling does not occur somatically more often in haploids or in hybrid diploids than in pure forms. Polyploid gametes are (on the whole) formed more frequently in hybrids than in pure forms, but, as I have shown, not as a direct result of this hybridity. Evidently therefore we may say that conditions affecting the origin of polyploids are unrestricted: conditions affecting their survival are, on the other hand, restricted by a great variety of circumstances affecting viability, fertility and constancy, and these all favour the hybridity of polyploid species (cf. Darlington, 1928).

(2) *Chromosome pairing and relationship.*

We are used to regarding the frequency of pairing of chromosomes in polyploids as well as diploids as a proximate indication of their material,

¹ The use of this expression seems to show that it means "somatic chromosome doubling."

and hence historical, relationships. Two recent series of observations especially seem to require a modification of this view. First we have the fact that chromosomes of *Primula floribunda* regularly pair with their homologues of *P. verticillata* in the diploid hybrid, but rarely, if ever, pair with them in the tetraploid, *P. kewensis* (Newton and Pellew, 1929). Conversely we have the occurrence of autosyndesis in "haploids" and hybrids of species in which autosyndesis does not normally occur. Secondly we have the fact that chromosomes of smaller size than the rest of the complement in certain circumstances in *Secale*, *Zea*, *Hyacinthus*, *Tradescantia*, *Matthiola* and *Fritillaria* (unpublished) pair less regularly at metaphase (cf. Darlington, 1929 b).

The general question is: how is it that chromosomes or chromatin elements which are capable of pairing and even pairing regularly, will, in certain circumstances, pair rarely or not at all? The further observations of the conditions of pairing in *Hyacinthus* suggest an answer. Pairing at metaphase depends on the formation of a chiasma between the chromosomes that have paired at pachytene. Chiasmata are usually formed at random and on the average in proportion to the length of the chromosome (where synapsis is complete). Three conditions may therefore make for failure of pairing at metaphase in chromatin material that is normally associated: (i) reduction in the length of the chromosome (fragmentation, as in *Secale*, *Tradescantia*); (ii) reduction of the length of the chromosomes which are strictly comparable and therefore capable of pairing (hybrids); (iii) competition in pairing in (a) a hybrid even-multiple polyploid (*P. kewensis*), or (b) a triploid (*Hyacinthus*).

The assumption of a structural hypothesis makes it impossible to deduce systematic conclusions from the observations of chromosome behaviour at one stage of meiosis, but at the same time puts some past difficulties in a clearer light. Pairing of chromosomes is no longer (as is often assumed, in hybrids of *Triticum* and *Nicotiana*, for example) a simple measure of the degree of their differentiation, but must be considered in relation to chromosome length, chiasma formation and the conditions of survival in a polyploid.

And it is in polyploids that this differentiation is of the greatest importance. Although prophase pairing has not been studied in hybrid polyploids, it would appear from the observations of pairing in tetrasomic *Hyacinthus* as well as on general grounds that structural differences between chromosomes (such as inversions and losses) would hinder pairing to a much greater extent than merely in proportion as the chromosomes were unlike, for mating at one point in a chromosome

leads to mating of the adjoining parts. Its efficiency should therefore be cumulative.

It follows therefore that the pairing of chromosomes within the haploid set of *Prunus domestica* (autosyndesis) does not argue against an adequate differentiation of the sets of eight in that species for giving regular formation of bivalents, but that, rather, in a species such as *P. domestica*, very slight differentiation is necessary to ensure regular bivalent formation on account of its one-chiasma habit. Similarly in *Digitalis*, *Crepis*, *Papaver*, *Betula*, *Rubus* and *Solanum*. Only in *Nicotiana* does autotetrasyndesis fail in a species of this type (Clausen and Mann, 1924; Brieger, 1928).

In a two-chiasma species however a disproportionately higher degree of differentiation will be necessary to ensure regular bivalent formation. In a polyploid of such a type autotetrasyndesis might well be expected to be incomplete. Only in the genus *Triticum* has this possibility been tested. In the "haploid" individual of *Triticum vulgare* ($6n$) no more than one or two pairs are formed (Gaines and Aase, 1926), while in the hybrid *T. spelta* ($6n$) \times *T. aestivum* ($2n$) from none to three of the seven supernumerary pairs of *T. vulgare* associate¹ (Kihara and Nishiyama, 1928). Expectation in respect of pairing is fulfilled, and this is an indication that differentiation of chromosome sets has followed the origin of the species and that the behaviour and morphology (and one might add genetic properties) of these sets at present can be little guide to the relationships of the species' ultimate diploid ancestors.

V. SUMMARY.

1. Perfect chromosome pairing can take place in a series of hybrids: *P. domestica* ($6n$)—*P. cerasifera* ($2n$)—*P. triflora* ($2n$)—*P. persica* ($2n$)—*P. Amygdalus* ($2n$). This proves that the haploid sets of these species are made up of groups of eight chromosomes which are homologous each to each. The chiasma type of association of the bivalent chromosomes is demonstrated (see diagram, Fig. 2).

2. The hybrid *P. domestica-cerasifera* behaves in every respect like a tetraploid species of *Prunus*. Thus the ultimate diploid ancestors of *P. domestica* pair in the hybrid (autotetrasyndesis). This and the formation of quadrivalents are expressions of the polyploid character of *P. domestica*.

¹ Although their presence interferes with the normal formation of the two chiasmata by the bivalents (compare Figs. 10–15 with normal meiosis in *Triticum*). In the same way in hybrid *Canna* Belling (1927) found that the small proportion of chromosomes which were paired, were associated at one end (by one chiasma) instead of at both.

The occurrence of autosyndesis in some species and not in others can be related to the circumstances of chiasma formation and the conditions of survival in a polyploid.

3. The abnormalities of meiosis leading to the formation of polyploid gametes are classified under four heads: (i) Nullification of the first or second division or both, which probably results from lagging of chromosomes. This occurs particularly in the hybrid *P. persica-Amygdalus*. (ii) The division of binucleate pollen mother-cells, an irregularity of somatic rather than meiotic mitosis, perhaps genetically related to male sterility (*P. avium*). (iii) Double division of unpaired chromosomes which probably results from lack of pairing. This occurs in *P. avium nana* along with the first two irregularities, which may thus lead to the formation of gametophytes with 96 chromosomes by a sporophyte with 24. (iv) Suppression of the first division, a result of complete failure of pairing alternative to (ii). The bearing of these observations on the theory of meiosis is discussed.

4. Some limitations to deductions of species relationships from chromosome behaviour are pointed out.

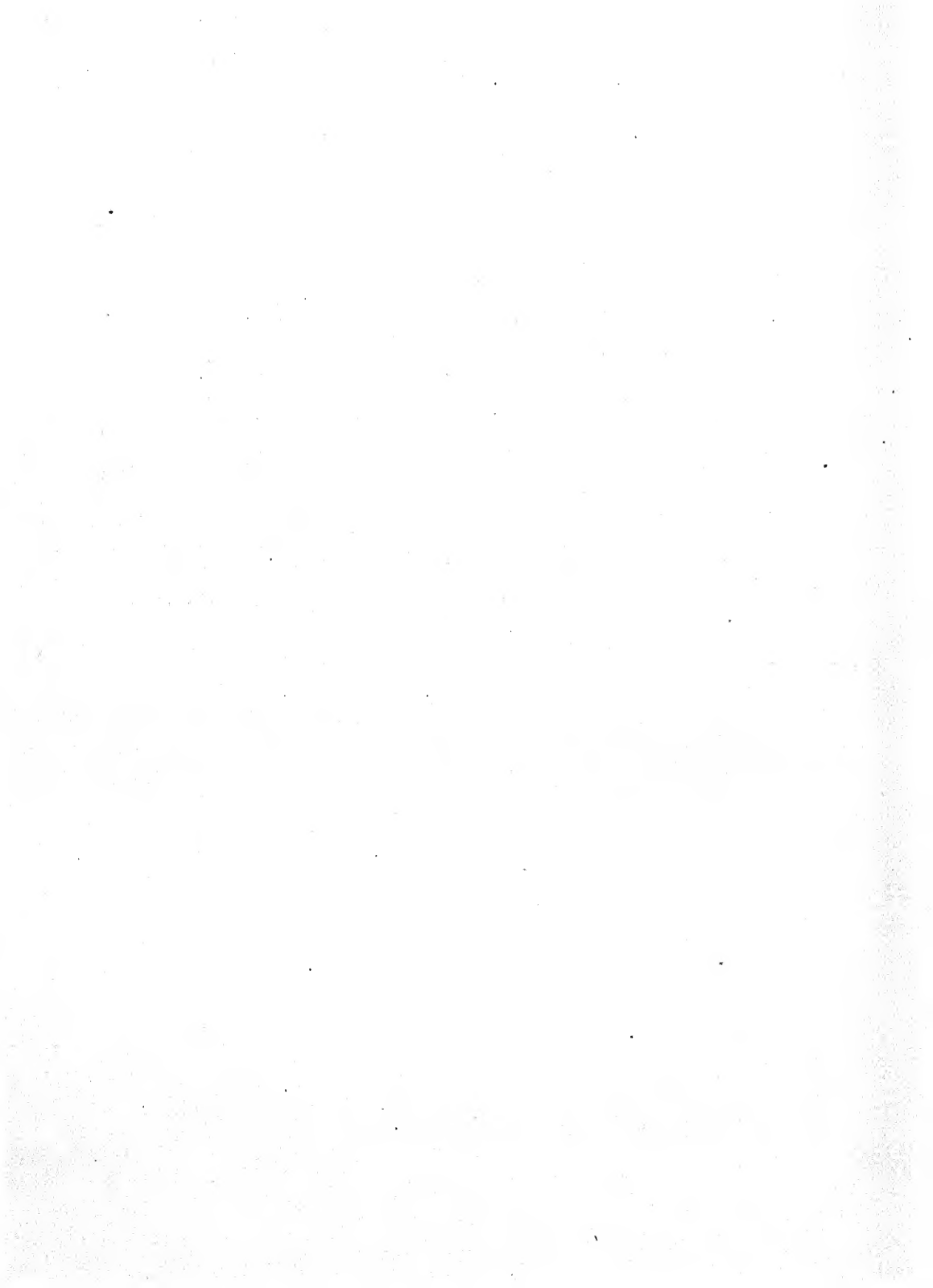
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FURTHER DATA ON A CASE OF AUTOSOMAL LINKAGE IN THE DOMESTIC FOWL.

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In a recent paper (Dunn, 1927) some evidence indicating the existence of a three-point autosomal linkage group in the domestic fowl has been published. The evidence was derived from the F_2 generation of a cross of single-combed White Leghorn by Silky fowls and the genes assumed to be linked were **I** (dominant white plumage), **he** (cerebral hernia), and **Po** (polydactyly). During the last three seasons we have been able to raise another F_2 generation from the same F_1 fowls which provided the data already reported, several back-crosses of heterozygous females by males recessive in **I** and **he**, and to observe the results of mating together the extracted herniated birds. The observations previously reported were made chiefly by one of us (L. C. D.) while the new observations have been made independently by the other (W. L.). The agreement between the two sets of observations is good, and the preliminary indications of linkage are sustained in the augmented data.

HERNIA.

Observations on the second generation.

Cerebral hernia, which was not observed in either of the parent types crossed, or in the F_1 , appeared in the F_2 generation. Out of 13 F_1 fowls tested by crossing *inter se*, 12 produced some herniated chicks, and it was assumed that all F_1 fowls were heterozygous in a recessive gene for hernia¹, and thence that the silky male used was homozygous for this gene, although he showed no hernia. Most of the F_2 hernia chicks previously reported were females, only two herniated males having been

¹ One F_1 ♀ (1132) produced no herniated chicks out of 21 observed. All other F_1 fowls tested (nine females and three males) produced herniated chicks. Some produced a very low proportion of herniated chicks, *e.g.* one female produced 37 normal and only two herniated when bred to a heterozygous male, indicating that although the gene was transmitted it was probably not expressed in many cases. In the case of the progeny of ♀1132 it is probable that its expression was entirely prevented.

observed, and there was some doubt about the sex records of one of these.

The new data establish beyond doubt that hernia appears in chickens of both sexes, but that it is more frequent in females than in males. The total F_2 generation consisted of 374 chicks, of which 317 were normal, while 57 (about 15 per cent.) were herniated. The degree of development of hernia varied considerably from a small low protuberance on the skull to a large, almost spherical hernia. Classification was made at hatching time from a careful gross examination and palpation of the skull, but it was evident that the condition might intergrade with normal. The sex distribution of the F_2 chicks was as follows:

	Normal	Hernia	% Hernia
Males	134	12	8.2
Females	153	39	20.3
Sex unknown	30	6	16.7
Total F_2	317	57	15.2

It is obvious that the character appeared more than twice as frequently in females as in males, and that it did not appear in a monofactorial ratio in either sex. The actual F_2 ratio was about 6 normal : 1 hernia.

Several crosses were made between F_1 and F_2 fowls heterozygous for hernia and the herniated fowls which appeared in F_2 . These produced 95 chicks of which 70 were normal and 25 showed hernia, a ratio of about 3 normal : 1 hernia. The same F_1 and F_2 fowls were crossed later with an F_3 male with pronounced hernia and produced 32 normal and 28 hernia chicks, a ratio which does not differ much from the 1 : 1 ratio expected. Finally matings have been made between F_3 herniated fowls and these produced 52 herniated and 9 normal chicks. All of the normals of which the sex record is available (6 in number) were males.

During the past season hernia has also appeared in the F_2 from a cross of a "creeper" male¹ with crest but no hernia by White Leghorn females. The F_1 consisted of crested and uncrested males and females but no herniated birds appeared. Four F_1 females by their F_1 brother produced 66 normal and 12 herniated chicks. The proportions of herniated progeny (15.4 per cent.) is very similar to the proportion noted in the F_2 from the cross of Silky by Leghorn.

These results may be explained as follows. The cerebral hernia which

¹ This male was descended from wild (probably feral) fowls brought from the Marquesas Islands and very kindly given to us by Mr H. M. Hubbard Jr. of Lyme, Conn. It can have no close relationship to any of our stocks.

appeared in our experiments depends chiefly on a single recessive gene. Our F_2 ratio resembles a monofactorial ratio more than any other type, while the back-cross evidence, especially the results of the back-cross to the F_3 herniated male indicates segregation of a single gene. Davenport (1906) has shown that a similar hernia found in Polish and Houdan fowls behaves as a single recessive to the normal condition, while Ghigi (1914) found that in crosses of herniated Polish by Game Bantams hernia segregated as a recessive but that its degree of development was lowered by the cross so that several generations of selection were required before the extracted hernia bred true to the type found in Polish. The facts in our case are similar to those reported by Ghigi except that in our experiments hernia was introduced from a normally non-herniated breed, the Silky. At its first appearance, *i.e.* in F_2 , its grade of expression was very low and it appeared in recognizable degree in only 60 per cent. of the chicks which were theoretically pure for the hernia gene. After selection for hernia the grade of expression increased so that in back-crosses of F_2 birds heterozygous for the gene by an F_3 herniated male 93 per cent. of the offspring pure for the gene developed hernia, while from matings of F_3 herniated birds 85 per cent. showed the character. We take this to mean that a gene for hernia occurs in the Silky breed but that in the absence of other factors necessary for its expression it seldom reaches a recognizable degree of development. After crossing with Leghorn, combinations of these other factors favourable to its expression occurred and these factors were accumulated by selection in the subsequent generations. One factor operating against the development of hernia is apparently the male sex environment, but the other factors favourable to hernia may apparently overcome this handicap and produce typical hernia in the male.

In our experience the expression of hernia is not only very variable in the embryo and newly hatched chick, but the character may become progressively less marked with advancing ossification of the cranium, especially in males. Thus males recorded as having pronounced hernia when hatched may show no protuberance at all as adults and may be classed as normals. A careful morphological study will be required to establish criteria for distinguishing low grade herniated from normal individuals at different stages of development. In our experiments we have been chiefly interested in the expression of this gene as a part of the experience required to determine its linkage relations.

Association with crest.

Both of the males in the descendants of which hernia was later found were crested and it is well known that many crested breeds (Polish, Houdan) are also herniated. Silky fowls, however, do not ordinarily develop hernia. Davenport found that "hernia is never found dissociated from crest," although his data contain some exceptions. Crest in his material was clearly dominant and hernia recessive and this is confirmed by the experience of subsequent investigators. In our material all herniated fowls reared to maturity have developed a pronounced crest. Nevertheless there is probably not a true genetic association or identity between crest and hernia, for we have found one instance in which an *uncrested* fowl has transmitted hernia. Since crest behaves regularly as a dominant this is good evidence that the two characters are separable in inheritance. The possibility of a close linkage between the two genes is made improbable by the fact that, although hernia is very closely linked with the recessive allelomorph of dominant white, crest appeared in our previous data to be independent of this colour gene. The possibility of a developmental association between the two genes remains to be investigated.

Colour.

The White Silky and White Leghorn varieties crossed were found to differ in two genes differentiating white from coloured plumage. The White Leghorn was IICCC^sC^s , while the white Silky was iiCCc^sc^s . Independent recombination of these genes produced in F_2 a ratio of 12/16 with gene **I** (dominant, epistatic white); 1/16 $\text{iiC(c)c}^s\text{c}^s$ (Silky recessive white, indistinguishable from the above), and 3/16 $\text{iiC(c)C}^s(\text{c}^s)$ (coloured). The phenotypic ratio in F_2 should thence be 13 white : 3 coloured, and the actual ratio for the whole F_2 agrees with this expectation:

	White	Coloured
Found	261	58
Expected	259.2	59.8

Linkage of hernia and colour.

Linkage (repulsion) between **I** and **he** was assumed from the fact that most of the white F_2 chickens were normal, while most of the coloured F_2 's were herniated. The complete F_2 data are given below, compared with the distributions expected if **I** and **he** segregate inde-

pendently, and if **I** and **he** are completely linked. In calculating expected ratios, the further assumption is made that half of the F_2 chicks homozygous for hernia have been classified as normal.

F_2 segregation of hernia and coloured plumage.

	White normal	White hernia	Coloured normal	Coloured hernia
Actual	247	14	24	34
Expected if I and he independent	222.1	37.0	51.3	8.5
Expected if I and he linked ...	249.2	9.9	29.9	29.9

The fit of the observed distribution to that expected if **I** and **he** are independent is so bad as entirely to eliminate this hypothesis. The distribution calculated on the assumption of complete linkage, however, fits the observed figures quite well.

A few crosses between F_1 and F_2 females which were heterozygous only in dominant white and hernia **I He/i he** and an F_1 male **I He/i he** yielded another small F_2 as follows:

White normal	White hernia	Coloured normal	Coloured hernia
34	0	6	8

This cross is not complicated by the occurrence of recessive white chicks, and it is obvious that no chick with dominant white plumage and hernia (cross-overs) appeared. The coloured normals as in the large F_2 are assumed to be due to non-expression of the hernia gene in about half the chicks which received it.

The back-cross data now available confirm the F_2 results. Five F_1 and F_2 females of genotype **I HE/i he** were mated with an F_2 coloured hernia male **iiheheCC**. Since the male was homozygous for the colour gene **C**, the only gene differentiating white and coloured was the dominant white gene **I** introduced by the female. These matings produced offspring as follows:

	White normal I He	White hernia I he	Coloured normal i He	Coloured hernia i he
Actual	40	1	20	22
Expected* (I and he independent)	31.1	10.4	31.1	10.4
Expected* (I and he linked) ...	41.5	0	20.7	20.7

* Assuming that half the **he he** progeny appear normal.

The actual result does not agree at all with that expected if **I** and **he** are independent. It agrees very well with that expected if **I** and **he** are very closely linked.

Another back-cross between F_1 and F_2 females of genotype **I He/i he** by an F_3 coloured herniated male (**iihehe**) produced the following progeny:

	White normal I He	White hernia I he	Coloured normal i He	Coloured hernia i he
Actual	29	3	4	24
Expected if I and he independent	15	15	15	15
Expected if I and he linked ...	30	—	—	30

The results of this cross, on account of the high degree of expression of hernia in the progeny, give a clear picture of close linkage. On account of the possibility that the coloured normal chicks may actually contain the hernia gene but fail to express it, the only certain cross-overs are the three white hernia chicks from this experiment and the single one of this type from the preceding back-cross or four probable cross-overs out of 143 gametes tested in both experiments, indicating a minimum cross-over percentage of between 2 and 3 per cent. These cross-overs in an autosome must have occurred in the oögenesis of a hybrid female, showing that in the fowl crossing-over does occur in the female. On account of the unverified assumption that all coloured normal chicks are genetically herniated this cannot be taken as a definitive measure of the linkage strength. Proof of the linkage itself, however, is not affected by the truth of this assumption, since, even if it is wrong, the cross-overs would still number, in the second back-cross, 7 out of 60 or about 10 per cent.

Linkage of polydactyly and colour.

The complete F_2 data also indicate linkage (repulsion) between polydactyly and dominant white. F_1 females heterozygous in both genes **I po/i Po** were crossed with an F_1 male heterozygous in dominant white but homozygous for the normal four-toed condition (**Iipopo**). Polydactyly segregated in a 1 : 1 ratio from certain of the females, while from others an excess of recessives was obtained, due probably to factors inhibiting polydactyly which have not yet been analysed. The segregation of polydactyly in the combined results resembles a 2 : 1 rather than a 1 : 1 ratio.

F_2 segregation of polydactyly and colour¹.

White 4-toed	White polydactyl	Coloured 4-toed	Coloured polydactyl
102	51	13	22

¹ Includes also two progenies from F_1 male by F_2 females heterozygous for dominant white and polydactyly.

If **I** and **po** are independent, the proportion of polydactyl and four-toed among the white chicks should be the same as among the coloured ones. This obviously is not the case, and there is an excess of white four-toed and coloured polydactyls, the non-cross-over classes. The exact cross-over ratio cannot be calculated from these results, but the linkage is apparently not as close as between **I** and **he**.

Linkage of polydactyly and hernia.

A similar departure from independent segregation is apparent in the case of polydactyly and hernia, for which the complete F_2 data are given below (from cross of F_1 ♀♀ — **He po/he Po** × F_1 male **He po/he po**).

F_2 segregation of polydactyly and hernia.

Normal 4-toed	Normal 5-toed	Hernia 4-toed	Hernia 5-toed
91	49	12	11

Whereas among the normals the four-toed outnumber the polydactyls by about 2:1, among the herniated chicks the four-toed and polydactyls appear in about equal numbers. Again the indication is one of loose linkage between hernia and polydactyly.

It is probable then, that the three genes considered are located in the same chromosome, **he** and **I** very near together, while **Po** is relatively distant.

Because of irregularities of expression of two of the genes concerned, this case offers rather difficult material for the investigation of autosomal linkage in the fowl but because autosomal linkages in the fowl have been so rare and especially since it apparently represents a three point linkage group, it should repay further investigation. Fortunately Serebrovsky and Petrov (1928) have recently reported linkage between two easily distinguishable dominant genes in another autosome which should provide much better material for the study of two point linkage phenomena.

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ON PIGMENT FORMATION IN THE D-BLACK RABBIT.

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THE mechanism of the origin and formation of pigment has been elucidated by the work of Bertrand (1908), Chodat (1912) and others. They all agree that colour in animals and plants is produced by the action of a ferment or oxidase substance on a colourless chromogen. Von Fürth (1904), Gortner (1911), Przibram, Dembowsky and Breecher (1915), Schultz (1925) and other researchers have demonstrated that the presence of an oxidase (tyrosinase) in the coloured tissues of animals and plants is a *conditio sine qua non* of pigment formation. L. Kaufmann (1923), for example, was able by injection of tyrosinase artificially to supply this oxidase and obtained the formation of melanin pigment.

Our present knowledge of the chemical basis of pigment formation allows us to use the chemical test for genetical analysis. In this connection the work of Onslow (1915) is of special interest. He demonstrated that chemical differences could be found between the two different types of white-coat colour in rabbits and mice, which correspond to differences in their genetic behaviour. In the dominant white coat the pigment-forming mechanism is present, but its action is inhibited by specific "inhibitor" substance. In the recessive white or albino, there is no inhibitor but the pigment-forming mechanism is incomplete.

Onslow used in his experiments the skin of recessive and dominant whites and of recessive black animals, as well as the chocolate form of the latter. He did not extend his work to dominant black, called by Punnett (1913, 1915) D-black, which is dominant to agouti, towards which the normal black is recessive. Our problem was to find out whether, an underlying chemical difference could be found corresponding to the different genetic behaviour of dominant black.

METHODS.

In the light of the more recent literature on oxidases and pigment formation (Schultz, 1925) Onslow's method with tyrosinase and peroxide seemed to be inadequate, and it appeared preferable to follow Bloch

(1917, 1924) in replacing these reagents with "Dopa" (1-3, 4, dioxy-phenylalanine).

As experimental animals 2-3 day old rabbits were used. The skin was carefully separated and freed from adherent connective tissue and blood vessels, washed with distilled water and rinsed and dried with a cloth. It was then cut into pieces and ground up in a mortar with pure sand which had been previously wetted with a few drops of water. The pulp was placed in a cloth and pressed out in a press. The juice was separated from solid particles by centrifuging. As material for dominant white or inhibitor, we used the belly of agouti and the white parts of English rabbits.

The extracts of skin were divided into equal parts, which were placed in weller china plates. In three of the extracts a small quantity of solid finely powdered Dopa was suspended. To one of these portions with Dopa a few drops of the extract of white skin from English rabbit or agouti belly was added. To another we added an equal amount of water to test the effect of dilution by water as compared with the effect of dilution by inhibitor extract.

The china plate was placed in a big Petri dish into which also wet cotton-wool was placed. The dish was covered and placed in a thermostat at 37° C. for 4 hours. Then the dish was kept for 8 hours at room temperature and the results noted. The extract of coloured skin alone (used as control) does not change its colour in this time, while the same with Dopa changes to a very deep black-brown colour.

DESCRIPTION OF EXPERIMENTS.

The following breeds were used as experimental material: (1) agouti; (2) recessive black; (3) dominant or D-black; (4) dominant white (homozyg. English rabbit); (5) recessive white or albino; (6) chocolate or dilute black; (7) agouti-Dutch.

1. *Extract of agouti skin.*

Extracts were prepared from the coloured coat of agouti and as inhibitor the extracts from the belly of the same agouti was used.

Extract	Reaction
<i>E</i>	-
<i>E</i> + Dopa	++
<i>E</i> + Dopa + H ₂ O	±±
<i>E</i> + Dopa + inhibitor	-

E = extract from coloured skin.

++ = positive reaction; formation of a precipitate of black colour.

- = negative reaction; no change in colour.

The reaction with Dopa and water was not so strongly positive as with Dopa alone, the water causing a dilution of precipitate colour. The result, however, shows that the agouti coat has all the factors for pigment formation, whereas the belly has in addition an inhibitor enzyme.

2. *Extract of recessive black skin.*

Extract	Reaction
<i>E</i>	-
<i>E</i> + Dopa	++
<i>E</i> + Dopa + H ₂ O	±±
<i>E</i> + Dopa + inhibitor	-

Two kinds of inhibitor were used: one was the agouti belly, in which case a negative reaction was observed. In crosses of agouti and recessive black the F_1 offspring have the dominant white belly. Similarly if we used the extract of dominant white as inhibitor, the reaction was again negative. In crosses of recessive black and dominant white the F_1 offspring are all white.

3. *Extract of dominant or D-black skin.*

Extract	Reaction
<i>E</i>	-
<i>E</i> + Dopa	+++
<i>E</i> + Dopa + H ₂ O	++
<i>E</i> + Dopa + inhibitor	++

The above result shows that the inhibitor (from agouti belly and dominant white) could not prevent pigment formation. Punnett (1915) suggests that in the D-black rabbit there is present an inhibitor enzyme which itself inhibits the action of the inhibitor from agouti belly and dominant white. Therefore when he crossed agouti with D-black in F_1 generation the white belly did not appear and the offspring were all black. In this case the chemical observation is in full agreement with the genetic behaviour.

4. *Extract of dominant white skin.*

Extract	Reaction
<i>E</i>	-
<i>E</i> + Dopa	-
<i>E</i> + Dopa + H ₂ O	-
<i>E</i> + Dopa + inhibitor	-

In order to test more exactly where lies the cause of pigment inhibition, extract from dominant white was used with Dopa. The result in every case was negative, except when the extract was boiled for a few minutes, when a melanin precipitation was observed. It is suggested that

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in that case the effect of inhibitor is destroyed by heat and the other components of the extract can, when combined with Dopa, produce pigment.

5. *Extract of recessive white skin.*

Extract	Reaction
<i>E</i>	-
<i>E</i> + Dopa	-
<i>E</i> + Dopa + H ₂ O	-
<i>E</i> + Dopa + inhibitor	-

Dopa cannot produce pigment in recessive white extract and even if we boil the extract the reaction remains negative. But if the extract from recessive black is added a positive reaction appears, because the lacking member of the pigment-forming series is supplied. If we cross, for example, albino (recessive white) with recessive black, the F_1 generation is black, because from the black parent the entire pigmentary system is introduced.

6. *Extract of chocolate skin.*

Extract	Reaction
<i>E</i>	-
<i>E</i> + Dopa	++
<i>E</i> + Dopa + H ₂ O	+
<i>E</i> + Dopa + inhibitor	-

The chocolate is a dilute black; in crossing its fate is the same. If we cross it with agouti, the white belly of the latter, which contains the inhibitor, will appear in F_1 .

7. *Extract of agouti-Dutch rabbit's skin.*

Three kinds of extract were made: E_1 , from the agouti coat; E_2 , from the white part of coat; E_3 , from the belly.

E_1 behaved in the same way as the agouti; *i.e.* with Dopa it gave a positive reaction. E_2 with Dopa did not give a melanin precipitation and this extract could not prevent pigment formation. E_3 had the same effect as the inhibitor from agouti. In crosses the behaviour of the white part is the same as that of recessive white, which shows that it lacks one member of the pigment-forming system.

RESULTS.

1. Extracts from the coloured coat of agouti, recessive black, chocolate and agouti-Dutch give strong melanin formation with Dopa.

2. Extracts from dominant white skin (belly of agouti, white parts of English rabbit) do not form melanin on addition of Dopa. If added to the extracts of the coat of coloured rabbits mentioned under (1), such an extract completely inhibits pigment-formation.

3. Extracts from the skin of recessive white rabbit (albino) do not form pigment with Dopa and are unable to inhibit pigment-formation of coloured skin extracts.

All these experiments are thus in full agreement with the results of Onslow.

4. Extracts of the skin of dominant black rabbits show a pigment formation similar to that observed in recessive black. If, however, an inhibitor, *i.e.* extract of dominant white skin is added; this is unable to inhibit the pigment formation.

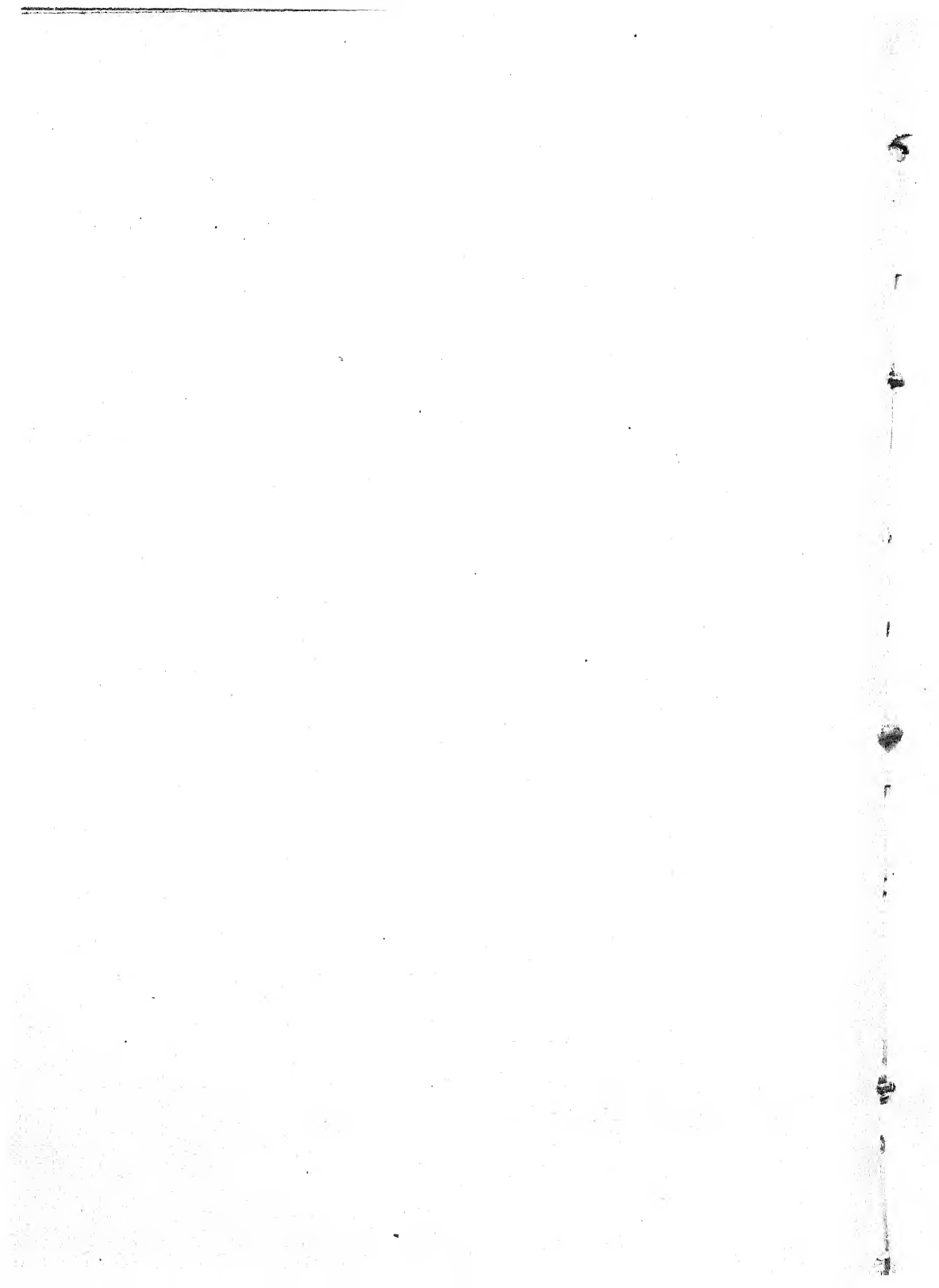
While Onslow's experiments thus adequately explain why albino is always recessive towards recessive black, and dominant white dominant to the same black, our observations give the chemical explanation of the dominant nature of D-black. In this case also the chemical experiments are in full agreement with the genetic behaviour.

It may be mentioned that in a few cases the inhibitor did not completely inhibit pigment formation in recessive black. In this case the animals were found to be heterozygous in regard to their inhibitor factor.

It is a pleasure to express my sincere gratitude to Prof. R. Crundall Punnett, F.R.S., for his suggestions and criticism as also for the supply of material for this study.

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THE GENETICS OF THE FOWL.

I. THE INHERITANCE OF FRIZZLED PLUMAGE.

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(With Two Plates and Two Text-figures.)

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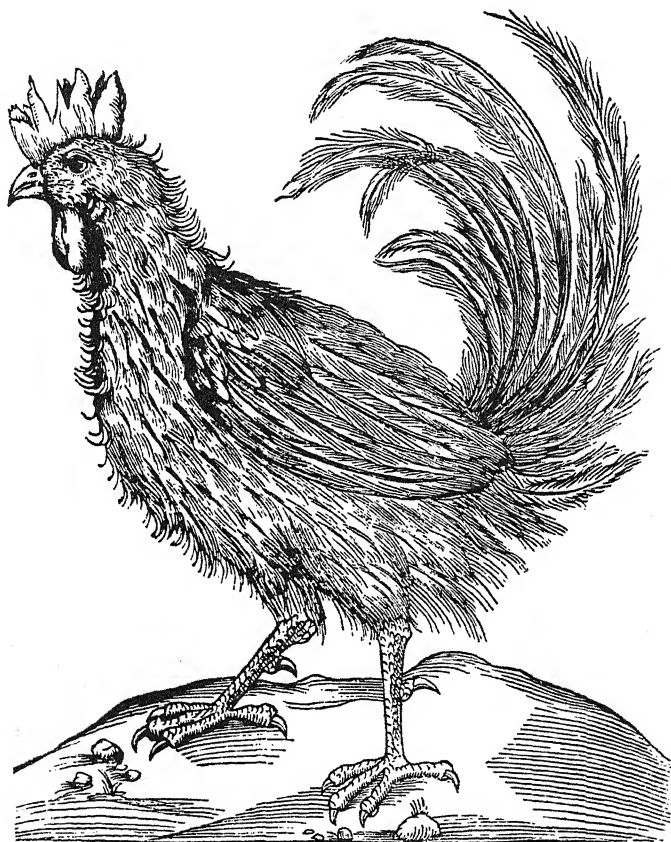
INTRODUCTION.

FRIZZLED fowls are neither very common nor of any special economic importance, but, as one of the most remarkable of the many varieties of the species, they are prized by fanciers in various parts of the world. For the geneticist they assumed a special interest when it was discovered that, in the experience of some breeders, they did not breed true to type, but always "threw" a certain proportion of normally feathered birds. Arising from this observation, the theory has become current that the homozygous Frizzle is non-viable and presumably dies at an early stage of embryonic development. The investigation herein reported was undertaken to substantiate or to disprove this theory and to determine the mode of inheritance of the character.

¹ The greater part of this investigation was conducted at the Animal Breeding Research Department, University of Edinburgh, in 1928, but the work was completed in 1929 at the University of Minnesota. This report is published with the approval of the Director as Paper No. 904 of the *Journal Series of the Minnesota Agricultural Experiment Station*.

HISTORICAL NOTES.

A frizzled fowl is illustrated (Text-fig. 1) in the classical work of Aldrovandus (1600), to whom a drawing and description of such a bird had been sent from Parma. His note concerning it, kindly translated for



Text-fig. 1. The frizzled fowl from Aldrovandus' *Ornithologiae* (1600).

the writer by Prof. J. B. Pike of the Department of Latin of this university, is as follows:

Pompilio Tagliaferro, the distinguished physician of Parma, has written to me with regard to this fowl as follows:

"I am sending you a drawing of a strange fowl; the artist, however, has by no means satisfied me with his representation. However, I wish you to note two noteworthy points discovered in regard to this fowl—things not seen in our cocks and hens. First

and most important, the feathers of the wings lie in a manner quite contrary to those in others, for the lower surface of the feathers, which ordinarily in other fowls turns inward, in this one curves out, with the result that the whole wing seems reversed. The second peculiarity worthy of note is that the feathers of the neck turn toward the head, like curls; the whole tail curves up in the same direction."

Such is his description. Neither the picture sent to me nor my own drawing brings out satisfactorily the peculiarities which he mentions. His words indicate that this was owing to the artist's lack of skill.

The variety was also known to Willughby, who, in his *Ornithology*, published in 1676, refers to its occurrence in England and, according to Tegetmeier (1867), states that it was at that time known as the Frisland hen

not because it was first brought to us out in Frisland, but because the feathers of the body are curled or frizzled; by which epithet I believe this bird was first called, the word being afterwards, by the mistake of the vulgar, corrupted into Frisland of like sound. For knowing it to be an outlandish hen, they thought it could not be more fitly denominated than from its country and thereupon imagined it to be called a Frisland hen, instead of a frizzled hen. Nor did they want a probable argument to induce them to think it to be of a Frisland breed or original, viz. the curling of the feathers which one would be apt to attribute to the horror of cold.

Linné (1758) calls the frizzled fowl *Gallus crispus* in the tenth edition of *Systema Naturae*, but in a later edition refers to it as *Gallus pennis revolutis*.

The frizzled fowl was known as "le coq frisée" to Buffon (1799) who states that it is to be found in Java, Japan and southern Asia. This range agrees with the statement of Temminck (ca. 1813, quoted by Tegetmeier, *loc. cit.*) that the "coq à plumes frisées" is kept under domestication in Java, Sumatra, southern Asia and the Philippines, the prevailing colour being white. Dürigen (1923) quotes the German ornithologist, Bechstein, as giving in 1793 a similar range with the addition of Surinam for its domestication, and states that it had also been reported by Captain Tollemache for Mauritius and Mozambique as the Hurricane Fowl.

The only published data concerning the inheritance of frizzling are those of Davenport (1906) who raised only one generation from a Silky \times Frizzle cross and found that the plumage of the latter variety was dominant. Of the 10 chicks raised, 6 were frizzled while 4 were normally feathered. Such a result would be expected if frizzling were caused by a single factor, and if some or all of the four Frizzles tested were heterozygous, but since the birds were not individually pedigreed no conclusions can be drawn other than that the character is dominant over normal plumage.

DESCRIPTION.

The peculiarity of the frizzled fowl is that the contour feathers, instead of conforming to the shape of the body, have their shafts so recurved that the outer surface of each feather becomes concave. The resulting general appearance is as if the feathers had been rubbed the wrong way (Plate III, fig. 2). In good specimens a prominent ruff is formed in the neck region. In the original flock used in this investigation the shafts of the rectrices and remiges were recurved only in the slender region at the tip of the feather, but in several parts of the vane in these large feathers small groups of the barbs, numbering from three to ten, were so twisted that they appeared to curl round the rachis (Text-fig. 2). In most cases these barbs were eventually worn off so that there remained only the shaft of the feather with small portions of the vane attached to it. This was particularly evident in the primaries. Because of this handicap the birds were unable to use their wings even as little as do other heavy domestic fowls, and had to be given low roosts to which they could easily hop. It is doubtful whether the mutation could persist long in a state of nature.

All of the 22 mature fowls with which the investigation was begun were fairly uniform in appearance, and conformed closely to the description given above. In the progeny of these birds, however, there appeared a distinctly different type of frizzling which will be described below.

Tegetmeier (*loc. cit.*) found in examining Darwin's collection of domesticated birds from all over the world that some specimens exhibited frizzling in all parts of the body, while in others it was confined to the neck region. The character is also found in the pigeon, where it has given rise to a variety known as the Frillback.

Frizzled fowls are found in various colours—black, white, buff and blue (dilute black), the chief preference of the fancier being for a bird all one colour and for uniformity in exhibition pairs, trios and pens. Those used in the present study were all red (*i.e.* as in the Rhode Island Red), excepting four bantams of which the cock was blue and the hens black, buff and blue.

There is some evidence that the frizzled fowls were originally black-skinned like the true Silkies of to-day. Marsden (1784), in his account of the island of Sumatra, refers to the domestic fowls, "some with black bones and some of the sort we call Friezland (*cf.* Willughby) or *negro fowls*." Darwin (1875) describes the Frizzled or Caffre fowls as being

"not uncommon in India, with the feathers curling backwards, and with the primary feathers of the wing and tail imperfect; *periosteum of bones black*." Dürigen (*loc. cit.*) gives the colour of the skin as one of the peculiarities of the variety, stating that it was originally dark red or purple, but that as a result of crossing with other fowls it has become light. This character has not been observed in any of the present writer's material, and there is no experimental evidence that it is linked with frizzling.

The writer was unable to detect any satisfactory indication of this type of feathering in the newly hatched chick, but frizzled birds could usually be positively identified as soon as the wing feathers had grown to half an inch in length or even less. In one case the classification of 47 chicks hatched at 6 days of age agreed exactly with a second classification at 14 days. Another lot of 66 chicks sorted out at 9 days was subsequently found when re-examined at 2 weeks to have been accurately classified on the first occasion. In some cases of extremely slow-feathering individuals the type of plumage could not be accurately decided upon till the chicks were over 2 weeks of age. In the data given below, only those chicks are included in which determination of the frizzled or normal type of feathering was definite. With the exception of birds dying early, all descriptions were re-checked at 2 weeks or later.

MATERIAL AND METHODS.

In the first year of the present study there were used 29 mature birds, of which 7 were normal and 22 were frizzled. All of the latter came directly or indirectly from the flock of Major G. S. Williams, Tredrea, Perranwell, Cornwall. Some of these had been purchased in 1927; a few were raised at Edinburgh that year, and 10 were loaned for the breeding season of 1928.

Matings in 1928.

In order to get sufficient numbers of chicks, incubation was begun early in February 1928, and chicks were hatched up to 26 July. Matings were made as follows:

Series A. Frizzle × Normal.

1. Eight frizzled females were mated with ♂ 283, a normally feathered cockerel extracted in 1927 from a Frizzle × Frizzle mating. One of these eight, ♀ 129, and another Frizzle, ♀ 132, had also been mated earlier in the season to a Brown Leghorn male, no. 142.

2. Two normal females, nos. 272 and 300, hatched in 1927 from a Frizzle \times Frizzle mating, were bred in separate pens to the frizzled cockerels P. 101 and M. 102 respectively. Both of these males had been hatched at Edinburgh in 1927 from a Frizzle \times Frizzle mating.

3. Three normal Bantam females were mated to a blue frizzled Bantam cock, B. 18.

Series B. Normal \times Normal.

1a. Two normally feathered females, nos. 272 and 300, were mated concurrently with Series A 1 to the normal male, no. 283. All three of these birds had been extracted from matings of Frizzle \times Frizzle.

Series C. Frizzle \times Frizzle.

1. Sixteen frizzled females were mated with the two frizzled cockerels, P. 101 and M. 102, each male having eight females in a separate pen. These matings were made in the same pens as those referred to in Series A 2 and concurrently with them. Ten of these females had previously been used in Series A 1, but care was taken to ensure that the influence of the normal male was lost before their eggs were included in Series C 1.

2. Three frizzled Bantam females were mated with the frizzled Bantam cock, B. 18, these birds being in the same pen as the normal Bantam females mentioned under Series A 3.

Incubation.

All eggs were incubated as nearly as possible in the same manner. The majority of them were started in a gas-heated Phipps incubator and moved to Hearson electric incubators a week before hatching. All hens were trap-nested, each egg was marked with the number of the hen laying it, and the chicks were hatched in pedigree bags, so that the ancestry of each chick was definitely known. The eggs were candled for infertility and for embryonic mortality at least twice during each hatch. Every dead embryo was examined and the estimated period of its death recorded, as well as any evidence of abnormality. Since there was a possibility that a lethal factor (if present) might be operative at an early stage of development of the zygote, all eggs which appeared infertile when candled were broken for more accurate observation, and when necessary an examination was made under the dissecting microscope.

RESULTS.

The results obtained in the three series of matings listed above are presented in Tables I, II and III. Since evidence of a lethal factor may

TABLE I.

Matings of Frizzle × Normal, 1928.

Series	Parents		Eggs set	In- fertile	Embryonic mortality in 2-day periods										Hatched		Chicks	
	♂ Normal	♀ Frizzle			1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-21	No.	%	Frizzle	Normal
A 1	283	50	15	—	—	—	—	—	—	—	—	—	—	5	9	60.0	6	3
	"	83	17	1	—	—	—	1	—	—	—	—	—	—	14	87.5	3	6
	"	128	10	—	1	—	—	—	—	—	—	—	—	1	7	70.0	2	3
	"	129	14	—	—	—	1	—	—	—	—	—	—	1	12	85.7	4	8
	"	134	10	—	—	—	—	—	—	—	—	—	—	—	10	100.0	7	3
	"	293	15	—	—	—	—	—	—	—	—	—	—	2	13	86.7	4	7
	"	301	16	2	1	2	—	—	—	—	1	—	—	4	1	7.1	1	0
A 2	"	398	19	1	—	—	—	—	—	—	—	—	—	1	16	88.8	5	11
	142	129	28	2	—	—	—	—	—	—	—	—	—	1	24	92.3	9	12
	"	132	12	2	—	—	—	—	—	—	—	—	—	1	8	—	4	4
	Frizzle	Normal																
A 3	P. 101	272	17	—	—	1	—	—	—	—	—	—	—	3	12	70.6	5	6
	M. 102	300	16	1	—	1	—	—	—	—	—	—	—	4	9	60.0	6	2
	B. 18	B. 10	13	1	—	—	—	—	—	1	—	—	—	1	9	75.0	3	4
	"	B. 63	10	—	—	—	—	—	—	1	—	—	—	—	8	80.0	3	4
	"	B. 394	9	1	1	—	—	—	—	—	—	—	—	7	87.5	2	3	
	Totals		221	11	2	11	3	2	2	1	4	—	2	24	159	—	64	76

TABLE II.

Matings inter se of normally feathered birds extracted from Frizzle × Frizzle matings, 1928.

Series	Parents		Eggs set	In-fertile	Embryonic mortality			Hatched		Chicks	
	♂	♀			1-10 days	11-18 days	19-21 days	Number	%	Frizzle	Normal
B 1	283	272	19	0	—	1	5	13	68.4	0	13
	283	300	19	0	—	—	2	17	89.5	0	14
Totals	—	—	38	0	—	1	7	30	—	0	27

TABLE III.

Matings of Frizzle × Frizzle, 1928.

Series	Parents		Eggs set	In-fertile	Embryonic mortality in 2-day periods												Hatched		Chicks	
	♂	♀			1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-21	No.	%	Frizzle	Normal		
C 1	M. 102	83	46	—	—	—	—	1	1	1	—	—	—	5	38	82.6	24	12		
	"	128	20	—	—	1	—	—	—	—	—	—	—	2	16	80.0	10	4		
	"	129	32	3	2	1	1	1	2	2	—	—	—	4	14	48.3	8	5		
	"	132	28	1	2	—	—	—	—	—	—	—	—	4	20	74.1	15	4		
	"	134	29	—	—	—	—	1	—	—	—	—	—	3	25	86.2	19	5		
	"	286	30	—	—	—	1	—	—	—	—	—	—	1	28	93.3	16	7		
	"	301	9	1	1	1	—	1	—	—	—	—	—	—	5	62.5	4	1		
	"	398	13	1	1	1	—	—	—	—	—	—	—	—	10	83.3	9	1		
	P. 101	50	17	2	1	1	—	1	—	1	—	—	—	—	10	66.6	5	2		
	"	293	10	—	1	1	—	—	—	—	—	—	—	3	6	60.0	4	1		
	"	341	37	1	1	1	—	1	—	—	2	1	—	6	24	66.7	12	11		
	"	342	46	1	1	1	—	—	—	—	1	—	—	7	34	75.5	22	10		
	"	343	13	5	1	3	—	1	—	—	1	—	—	1	2	25.0	2	0		
C 2	"	344	33	—	1	—	2	—	—	—	1	—	—	4	25	75.7	14	9		
	"	345	41	—	—	—	—	—	—	—	—	—	—	7	34	82.9	25	5		
	"	346	27	1	3	—	—	—	—	—	1	—	—	2	20	76.9	12	4		
	B. 18	Black	26	—	—	—	1	—	—	—	—	—	—	7	17	73.1	9	7		
	"	Blue	20	—	—	—	1	—	—	1	—	—	—	3	16	80.0	9	4		
	"	Buff	22	1	1	1	—	—	—	—	—	—	—	2	17	80.9	11	4		
	Totals		499	17	13	11	7	7	6	4	10	1	0	62	361	—	230	96		

be sought in the rates of embryonic mortality, as well as in the ratios of the two types of feathering in the progeny from each hen, the incubation records and the classifications of progenies are given in detail for each hen in each mating. Any differences between the numbers of chicks hatched and of those classified from the same individual indicate chicks dying too early to be classified. The percentage hatch is calculated for the fertile eggs only.

INTERPRETATION OF 1928 DATA.

A. *Matings of Frizzle × Normal.*

On reference to Table I it is seen that in Series A every one of the 13 frizzled birds tested, whether male or female, produced both frizzled and normal offspring in approximately equal numbers when mated to normal fowls. (♀ 301 had only 1 chick in this series but was subsequently shown in Series C to be heterozygous.) The total numbers in the two classes were 64 Frizzles to 76 Normals. The deviation from the 1 : 1 ratio expected, if all the Frizzles tested were heterozygous in the pair of factors affecting frizzling (Table IV), is only 1.47 times the probable error of the ratio and may therefore be considered insignificant.

The results in Series A may therefore be construed as indicating:

- (1) That frizzling is a dominant character dependent for its expression upon a single pair of factors.
- (2) That the 9 females and 3 males tested were all heterozygous with respect to that pair of factors.
- (3) That no sex-linked genes are involved, since the crossing of frizzled males with normal females gave results similar to those in the reciprocal cross.

B. *Matings inter se of extracted normals.*

In conformity with the results obtained in Series A it was found (Table II) that breeding together normally feathered birds extracted from Frizzle × Frizzle matings produced only normals and hence that these were true simple recessives.

C. *Matings of Frizzle × Frizzle.*

In this series (Table III) normally feathered chicks were obtained from every one of the 19 hens, 10 of which had not previously been tested. Since all of these Frizzles were thus shown to be heterozygous, the expectation in their progeny (considering the findings in Series A) was a

simple 3 : 1 ratio, provided no lethal factors are involved. While the deviation of 14.5 from expectation (Table IV) seems fairly large for a population of 326 individuals, it is only 2.75 times the probable error of the ratio, and is therefore well within the limits of fluctuations due to sampling.

TABLE IV.

Ratios obtained in Series A, B and C compared with those expected on the basis of frizzling being a unifactorial dominant character either lethal or non-lethal in the homozygous condition.

Series		Frizzle	Normal	Deviation	Probable error	Deviation P.E.
A	Observed	64	76	6	—	1.47
A	Expected (1 : 1)	70	70	—	±4.08	—
B	Observed	230	96	14.5	—	2.75
B	Expected if FF were non-lethal (3 : 1)	244.5	81.5	—	±5.27	—
B	Expected if FF were lethal (2 : 1)	217.3	108.7	12.7	±6.08	2.09
C	Observed	0	27	—	—	—
C	Expected	0	27	—	—	—

At the same time it should be pointed out that in both Series A and Series C there is a deficiency of frizzled chicks. In fact the ratio in the latter series fits a 2 : 1 expectation equally as well as one of 3 : 1 (Table IV).

The deficiency of Frizzles in Series C results from an extreme deviation from a 3 : 1 ratio in the progeny of certain females mated to ♂ P. 101 and ♂ B. 18. (The progenies from ♂ M. 102 make up an almost perfect 3 : 1 ratio, there being 105 Frizzles to 39 Normals where the expectation is 108 : 36.) Of the hens mated to ♂ P. 101, nos. 341, 342 and 344 gave deviations from the expected ratio so marked that they might be considered as indicating a differential production of gametes by ♂ 101, were it not for the fact that by the same sire ♀ 345 produced an excess of frizzled chicks and ♀ 346 an exact 3 : 1 ratio. Moreover, it had been shown in Series A 2 (Table I) that ♂ P. 101 was producing the two classes of gametes in equal proportions.

The use of the χ^2 test for goodness of fit of Mendelian ratios was formerly confined to polyhybrid ratios, but Kirk and Immer (1928) have recently shown that it may be applied equally well to a monohybrid ratio, where several progenies are under consideration. By its use the deviations from the expected ratio in each progeny are considered, and one obtains a more accurate conception of the validity of an observed ratio than is possible by the use of the probable error, when the latter is applied to the ratio obtained by the summation of the class frequencies in all progenies.

When the χ^2 test, using the tables of Fisher (1928), is applied to the data in Table III, it is found that $\chi^2 = 19.345$, $n = 19$, $P = 0.44$.

In other words, a deviation from an expectation of 3 : 1 as great as that observed in the ratio of 230 Frizzles : 96 Normals would be expected in about 44 per cent. of similar trials. It is, therefore, quite insignificant and the hypothesis that the homozygous Frizzle is viable is supported by the ratio obtained.

Embryonic mortality.

It was more difficult to explain why each of the 22 frizzled birds tested in this investigation and some, at least, of the four used by Davenport (*loc. cit.*) should have been heterozygous. Since one would expect that in an unselected population of this size considerably more than one-third would be homozygous, there was every reason to suggest that a lethal factor is involved.

Such a lethal might be either gametic or zygotic. The possibility of a gametic lethal is eliminated by the results in Series A of this experiment (Table I), which showed that 10 females and 3 males were producing viable gametes carrying the factor for frizzling in the same proportion as gametes carrying the recessive factor.

If a zygotic lethal were involved, it might be operative either during embryonic development or at any time after hatching. Consideration of the time of effect of lethals in other animals, and of the few known in fowls, leads one to suspect such a factor to be effective during the period of incubation. The percentage mortality in each four-day period of incubation, for all eggs set in Series A and C, is shown in Table V.

TABLE V.

Comparison of embryonic mortality in Series A and C.

Series	Mating	Fertile eggs	% mortality in 4-day periods					Total mortality	Hatch %
			1-4	5-8	9-12	13-16	17-21		
A	Frizzle \times Normal	210	6.19	2.38	1.43	1.90	12.38	24.28	75.71
C	Frizzle \times Frizzle	482	4.98	2.90	2.07	2.28	12.86	25.10	74.89

If homozygosity for frizzling were lethal to the embryo, a peak of mortality would be expected at some stage of incubation for the eggs set from matings of Frizzle \times Frizzle. In addition one would expect approximately 25 per cent. higher mortality in this series than in eggs from matings of Frizzle \times Normal. Neither of these conditions was

present. The death rate in one series ran closely parallel to that in the other. The peak of the mortality during the last five days is quite usual, and to a considerable extent is due to fully formed chicks dying from being in positions which make it impossible or extremely difficult for them to hatch.

Embryos representing various types of teratological monsters, chondrodystrophic chicks and abnormalities in position were found, but in no greater numbers in one series than in the other, and in no greater proportion altogether than in a large number of eggs from other sources.

In view of these findings it seems reasonable to conclude that homozygosity for frizzling is not lethal to the embryo.

Reason to doubt the validity of this conclusion is found (Table III) in the fact that from the 8 females with less than 75 per cent. hatch the proportion of frizzled to normal chicks was only 59 : 31 (*i.e.* 2 : 1), while from the 11 fowls with more than 75 per cent. hatch the proportion was 171 Frizzles : 65 Normals, a fairly close fit to the expectation of 3 : 1. At first sight this association of a deficiency of Frizzles with the poorer hatches certainly suggests that a lethal factor is involved. However, further examination shows that the 2 : 1 ratio in the progeny of the first class arose from the inclusion therein of ♀ 341 and ♀ B. Black, both of which gave extreme deviations from a 3 : 1 ratio and whose offspring made up 39 of the 91 chicks in this class. The other 6 hens of this class all had ratios closely fitting the 3 : 1 expected, but not enough chicks to offset the influence of the two exceptional progenies on the totals.

Similar fluctuations are found in the group of hens with hatches over 75 per cent., where ♀ 83 had an exact 2 : 1 ratio (out of an 82 per cent. hatch) and ♀ 344 produced only 14 Frizzles to 9 Normals. In this class, however, there were enough large-sized progenies nearer to the expected ratio to make the totals for the whole class fit fairly close to a 3 : 1 ratio. The difference between the proportions of frizzled to normal chicks in the group with hatches under 75 per cent. and in those over 75 per cent. may therefore be considered a coincidence and not owing to the action of some lethal factor linked with the gene for frizzling.

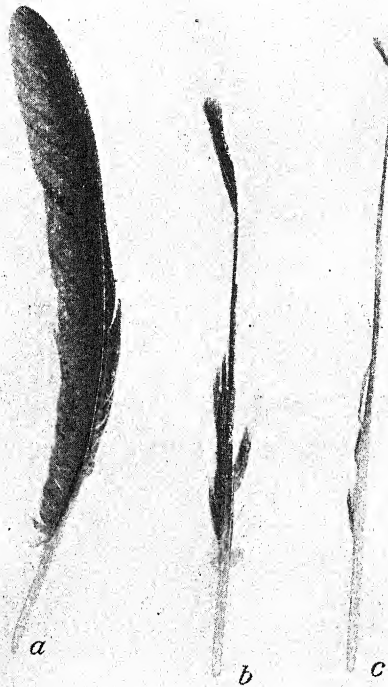
The possibility that the homozygous individual may be killed off at some time after hatching was entertained but was later discarded for reasons given below.

"EXTREME" FRIZZLING.

A clue to the whereabouts of the lost homozygous Frizzle appeared when the chicks hatched in 1928 began to assume their definitive plumage.

It became evident that there was an extreme type of frizzling not present in any of the breeding stock from which the chicks had sprung.

In this extreme type the body feathers have not only the rachis extremely recurved but also the barbs so curled that not a feather on the body has the normal flat vane. As a result the general appearance of



Text-fig. 2. Primaries from (a) a Rhode Island Red, (b) a heterozygous Frizzle, and (c) a homozygous Frizzle. All are from the left wing and from females of comparable size.

the bird is woolly (Plate III, fig. 1) rather than frizzled. Microscopic examination has shown that the absence of the normal vane of the feather is not caused, as in the Silky, by the extreme reduction, or absence from the barbules, of the barbicels and hamuli which ordinarily lock the barbs together. It arises from the extreme curling of the barbs, and even of the barbules, so that only a few of the former can remain fastened together. The shafts of the rectrices and remiges are curled

more than in the other type of frizzling, and their barbs are so curled that very little normal vane is formed. The barbs are quickly worn off the primary feathers. A comparison of the feathers in the extreme type, in the ordinary frizzle and in the normally feathered fowl is shown in Text-fig. 2 and in Plate IV, fig. 3.

By the time this new type was recognisable a number of the chicks had died, and others had been marketed. Some were not sufficiently grown to be classified as of the extreme type or as ordinary Frizzles by the time the writer left Edinburgh. However, a classification was made on 16 September, 1928, of 70 frizzled chicks, including all those which were at that time mature enough to be described as belonging to one type or the other. Pedigrees of these chicks were unknown at the time of classification so that, although it was in some cases difficult to place a bird in one class or the other, personal bias was entirely eliminated. Subsequently the pedigree of each chick was traced.

TABLE VI.

Classification of a random sample of frizzled chicks raised in 1928.

	Extreme frizzling	Ordinary frizzling
From matings of ordinary Frizzle \times Normal	0	4
<i>Expected</i>	0	4
From matings <i>inter se</i> of ordinary frizzles	25	41
<i>Expected if extreme Frizzles are FF and ordinary Frizzles Ff</i>	22	44

The records (Table VI) show that the proportion of extreme to ordinary Frizzles among chicks from Frizzle \times Frizzle matings was quite close to the proportion of dominant homozygotes to heterozygotes expected in a random sample of the F_2 from a monohybrid cross. The assumption was therefore warranted that the extreme individuals were the lost homozygous Frizzles and that the ordinary Frizzles were always heterozygous.

This view was strengthened when Major Williams wrote that it was his policy "to discard in all cases the narrow feathered and unevenly feathered birds." He stated further that, with his Bantams, mating of plain cock with frizzled hens, or the reciprocal cross, produces frizzled birds having the quality of feather and curl desired, whereas mating frizzled birds together produces "perhaps eighty per cent. Frizzles and of these about three-quarters are narrow feathered and useless."

These facts fit perfectly with the hypothesis that the homozygous frizzled fowl is viable but is discarded by the breeder, with the exception

that one would expect about one-third rather than three-quarters of the last class mentioned to be undesirable. The difference probably indicates a selection for the desired show type so rigid that it excludes not only the homozygous individuals but some of the heterozygous ones as well.

In consideration of all these facts, it is reasonable to assume that the reason why no homozygous frizzled fowls were present among the 22 birds tested in the first year of this investigation was that these birds constituted not a random sample but a population of individuals selected according to a breeder's standard which, presumably, barred out the homozygous specimens.

MATINGS IN 1929.

Fourteen frizzled fowls, offspring of Frizzle \times Frizzle matings, were brought to the University of Minnesota in December 1928. One of these was killed by a rat, one died of diphtheritic roup, and another was killed after a prolonged attack of paralysis. The remaining 11 fowls (all females) were mated in March 1929 to a White Leghorn cockerel. One of them (with ordinary frizzling) had no hatchable eggs among the 14 set, and died in April, apparently as a result of having become crop-bound.

Of the 10 Frizzles which produced chicks, 5 exhibited extreme frizzling and 5 were of the ordinary type. Their progenies, shown in Table VII, indicate clearly that, as was anticipated, the five of the extreme type were all homozygous (FF) while the ordinary type were all heterozygous (Ff).

TABLE VII.

Progenies from frizzled females \times White Leghorn male (ff), 1929.

	Classification of progeny		
	Extreme frizzling	Ordinary frizzling	Normal feathering
Ordinary Frizzles (Ff)			
A 1	None	6	10
A 2	"	6	3
A 7	"	5	8
A 11	"	14	6
A 14	"	2	2
Total	None	33	29
Expected	None	31	31
Extreme Frizzles (FF)			
A 3	None	1	None
A 5	"	15	"
A 8	"	16	"
A 9	"	12	"
A 10	"	4	"
Total	None	48	None
Expected	None	48	None

DISCUSSION.

The case of the Frizzle is thus shown to be parallel to that of the Blue Andalusian. In both varieties the phaenotype preferred by the fancier is heterozygous and so cannot breed true to type, but fowls of the kind desired for exhibition can be secured by mating together the two rejected phaenotypes.

The use of the White Leghorn male in the 1929 matings provided an opportunity of observing the effect of combining the sex-linked gene for rapid feathering, carried by that breed, with the autosomal gene for frizzling. The writer ventures no definite opinion concerning the manner of action of the gene for frizzling, but it was considered possible that it might cause one side of the feather follicle to grow more rapidly than the other—the same condition as that causing the curling of hair. If that were the case, the gene *s* for rapid feathering might be expected to produce upon frizzling an effect different from that of its allelomorph *S* for slow feathering. Both members of this pair obviously influence the rate of growth of feather follicles.

As was expected, the White Leghorn male proved to be of the constitution *ffss*. The females were of the following genotypes: 3 *FFs*-, 1 *Ffs*-, 2 *FFS*- and 4 *FfS*-.

Figs. 6 and 7 (Plate IV) show that in both rapid-feathering and slow-feathering frizzled chicks the tips of the feathers are recurved to about the same degree. However, 3 of the 15 chicks (all rapid feathering) from ♀ A. 5 exhibited only a slight degree of frizzling. In one case (Plate IV, fig. 5) there was doubt as to its correct classification till it was found that the barbs of the primaries were curled as in the more obvious specimens. Since only three of these appeared, it is likely that this phaenotype arose from interaction of the gene *F*—not with the gene *S* or its allelomorph—but with some other unknown factors influencing feather growth. The differences commonly observed between rates of feathering of individuals within a flock of which all members are slow-feathering indicate that such factors exist.

During the breeding season the feathers of the Frizzles become broken in most specimens so that the birds appear half naked. This is particularly the case with the homozygous fowls. In July, after the males had been with the flock four months, it was quite easy to sort out the five homozygous hens from the rest of the flock since the former were all nearly naked (Plate IV, fig. 4) while the others were in much better feather. This condition did not result from precipitate moulting but from the

breaking of the feathers, usually quite close to the follicles. This was not confined to the back and head, but also occurred to a lesser extent on the breast and on the outer surface of the neck. It was apparently not all due to the treading by the male. In some cases (Plate IV, fig. 4) only the barbs were broken so that the shafts remaining gave some of the pterylae an appearance of being spiny rather than feathered.

Breeders' opinions concerning the hardness of frizzled fowls, as given by various poultry books, are controversial, but the majority of them rate the Frizzle as rather delicate and unsuited to cold climates. Buffon (*loc. cit.*) says "ce coq appartient plus particulièrement aux pays chauds, car les poussins de cette race sont extrêmement sensibles au froid et n'y résistent guère dans notre climat."

Sir Claude Alexander, Bart., writing of *Frizzles* in the *Feathered World* (London) of 12 April, 1929, says, "The first difficulty to a beginner lies in the three types of plumage, the correct frizzle, the overdone nearly naked curly and the smooth.... Kill the 'curlies'; they cease to thrive as soon as they shed their down and cannot survive our winters."

The writer's observations are not in accord with those just given in these two quotations. The 4 mature frizzles which have died since their arrival in Minnesota were all of the ordinary (heterozygous) type. The remainder, half of which were homozygous (*i.e.* "curly") spent the winter in a well-built but unheated pen while the temperature outdoors ranged as low as 24° F. below zero, *i.e.* 56° below freezing. The mean temperatures (Fahr.) here for the months of January and February, 1929, kindly furnished by the United States Department of Agriculture Weather Bureau at St Paul, were as follows:

	Mean daily minimum	Mean daily maximum	Mean daily mean
January	-6.1°	9.9°	1.9°
February	2.1°	17.2°	9.6°

Under these circumstances the conclusion is justified that both types of Frizzles are quite viable and able to withstand exceedingly cold weather, at least when kept dry. Furthermore, the fact that homozygous and heterozygous fowls were found in the expected proportions in a random sample of the population at Edinburgh (Table VI) indicates that the survival rate of the homozygotes is quite as good as that of the heterozygotes.

SUMMARY.

1. An investigation has been conducted to determine the mode of inheritance of frizzling in the domestic fowl and to substantiate or disprove the current theory that homozygosity for frizzling is lethal.

2. Two types of frizzling, ordinary and extreme, are differentiated.

3. Incubation records failed to show any evidence of a zygotic lethal factor being operative during embryonic development. It was found that no gametic lethal factors are involved.

4. Mating of ordinary Frizzles to normally feathered fowls produced the 1:1 ratio of parent phaenotypes expected in a back cross to a recessive. Offspring from matings *inter se* of normally feathered fowls extracted from matings of Frizzle \times Frizzle were all normal.

5. The progeny from matings *inter se* of ordinary Frizzles gave a 3:1 ratio of Frizzles to Normals and in a random sample of this population the ratio of extreme to ordinary Frizzles was as 1:2.

6. Five extreme Frizzles were tested and proved to be homozygous for the character, producing, when mated with a normally feathered male, only chicks showing the ordinary type of frizzling.

7. Eighteen ordinary Frizzles were tested and found to be heterozygous.

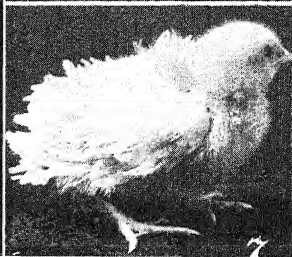
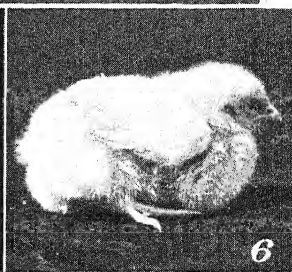
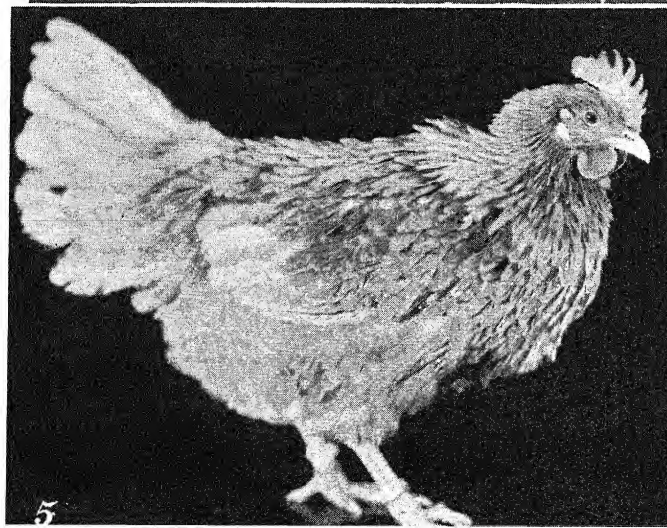
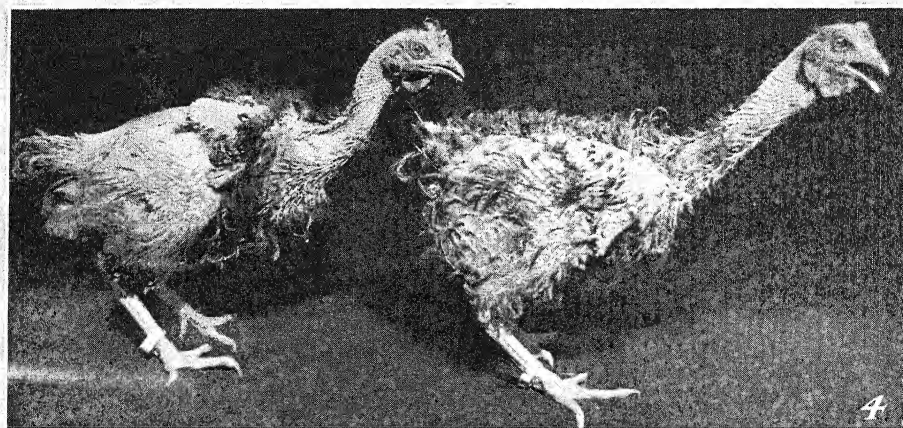
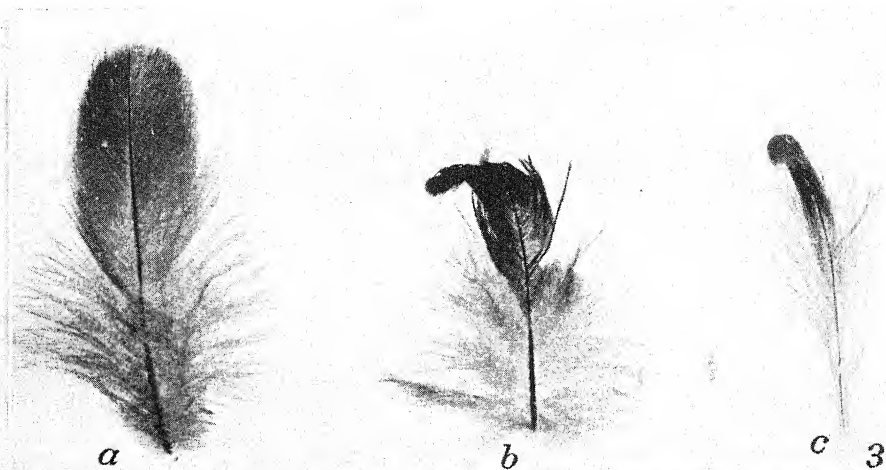
8. Reciprocal crosses showed that the character is not sex-linked.

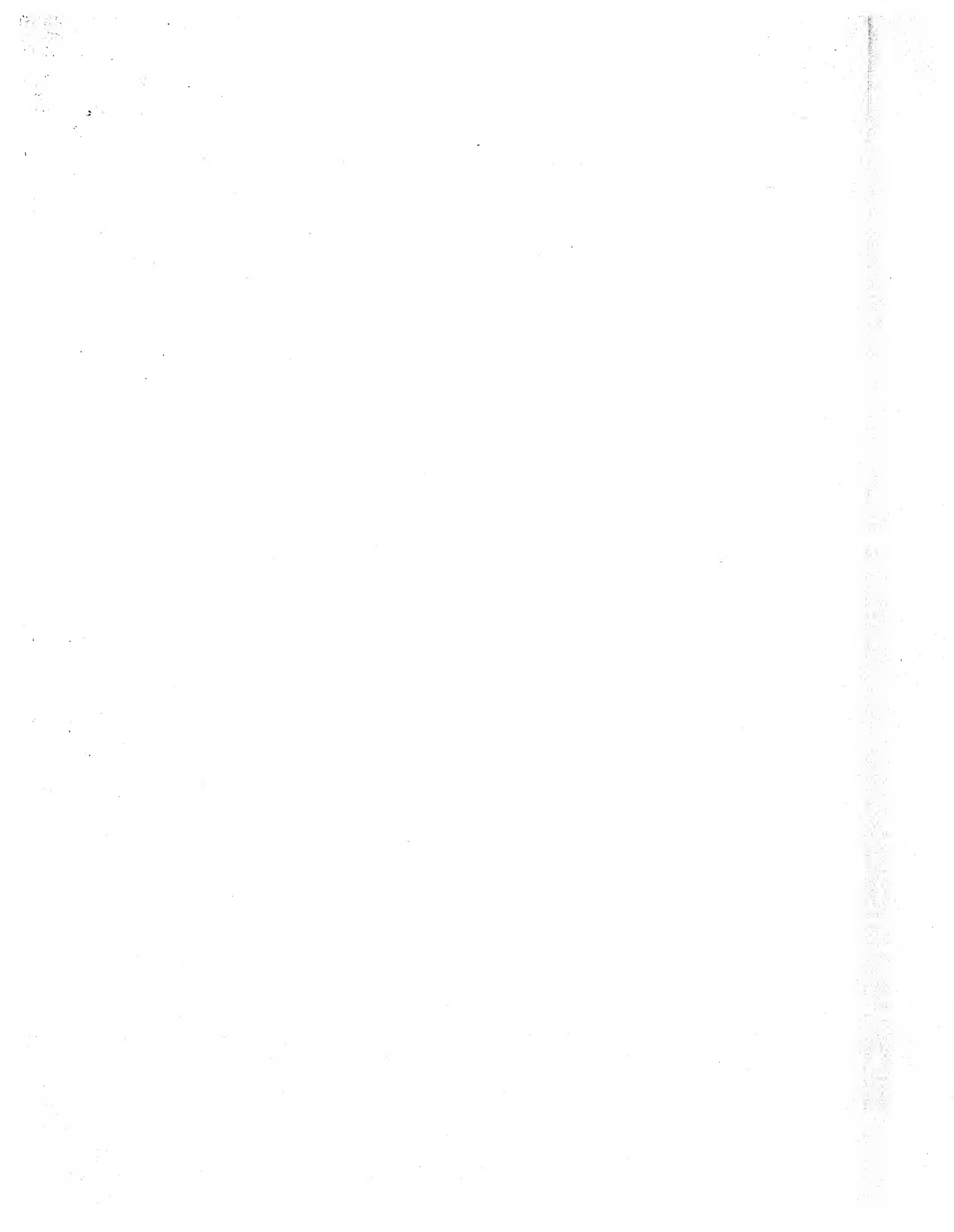
CONCLUSION.

It is concluded that frizzling is a dominant character, the expression of which is dependent upon a single autosomal pair of factors. The homozygous individual exhibits a more extreme type of frizzling than does the heterozygous one. The reputed inability of Frizzles to breed true arises from a preference by the fancier for the phaenotype exhibited by the heterozygous fowl, resulting in the exclusion from the breeding pen of all those that are homozygous for frizzling. Both genotypes are quite viable.

ACKNOWLEDGMENTS.

The writer wishes to express his indebtedness to Major G. S. Williams, Tredrea, Perranwell, Cornwall, who not only supplied valuable data concerning his own flock but also, at his own suggestion, very generously loaned ten Frizzles for the entire breeding season. Thanks are also due to the Animal Breeding Research Department, Edinburgh, for birds and facilities placed at the writer's disposal, and to Prof. F. A. E. Crew for suggestions and advice.





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EXPLANATION OF PLATES III, IV.

PLATE III.

- Fig. 1. A Frizzle of the extreme or "curly" type, homozygous for the character.
- Fig. 2. A Frizzle of the ordinary type preferred by the fancier, heterozygous for the character.

PLATE IV.

- Fig. 3. Feathers from the wing bow of (a) a Rhode Island Red, (b) a heterozygous Frizzle, and (c) a homozygous Frizzle. All are from females in definitive plumage.
- Fig. 4. Two homozygous female Frizzles photographed 18 July, 1929, at the end of the breeding season. The spiny appearance of the ventral pteryla in the lower neck region, caused by the feathers losing their barbs, is evident.
- Fig. 5. One of three fowls, from a homozygous Frizzle ♀ × White Leghorn ♂, exhibiting only a slight degree of the character.
- Fig. 6. A 14-day male chick heterozygous for frizzling and for slow feathering (Ffs).
- Fig. 7. A 14-day rapid-feathering female chick heterozygous for frizzling (Ffs-).

PRIMARY AND SECONDARY CHROMOSOME BALANCE IN *PYRUS*.

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(With One Plate and Forty-one Text-figures.)

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I. INTRODUCTION.

EARLIER workers have shown that seventeen is the haploid number of chromosomes in the genus *Pyrus* (including *Malus*) and that cultivated forms are diploid and triploid. There are, however, numerous discrepancies in their accounts, owing partly to the different methods they have used. The numbers given by those who have examined only the pollen mother-cell divisions (Shoemaker, 1926; Kobel, 1927; Heilborn, 1928) are often lower than those given by authors who have examined the root tips as well (Rybin, 1927; Nebel, 1929¹). This, we believe, is due to the occurrence of multiple association of chromosomes in *Pyrus*, where polyploidy was not suspected. These associations have often been misinterpreted or neglected.

¹ Sax (1929) has also reported 17 bivalents at meiosis in *Pyrus* hybrids.

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The number seventeen in itself is an anomaly in the Rosaceae, where seven and eight have been generally found. The "intragenomatische Affinität" assumed by Nebel, who has done the most important work on the genus, appears to us to throw some light on the relationship of this set of seventeen with the smaller series, but it was interpreted by him in quite another way¹. While disregarding the evidence of chromosome behaviour at meiosis, Nebel advanced the hypothesis that *Malus* species were halved pentaploids derived from forms with a basic number of seven. This hypothesis is without precedent and is put forward without the necessary evidence.

Again, Nebel observes that "die dreifache Bindung homologer Chromosomen kann durch autosyndetische Bindung von 51 sich frei paarenden Chromosomen vertreten werden." This is contrary to our view that the pairing of chromosomes is a criterion of their homology, being conditioned by the identity of their parts. It would seem to be a relic of the telosynaptic fallacy of the pairing of non-homologous chromosomes, which we may now happily discard.

The present study is an attempt to put these questions of chromosome behaviour and relationship in a clearer light and so make possible a correlation of genetical and cytological observations in this important group.

II. MATERIAL AND METHODS.

We have examined material of three species, *Pyrus Ringo* L., *P. floribunda* Kirchn. and *P. Malus* L., including thirty-one varieties and seventeen seedlings of cultivated apples. We were able to study root tip preparations of these varieties; rooted cuttings were kindly provided for this purpose by the Director of the East Malling Research Station. Previous workers have had to depend for evidence of chromosome number on the flower buds of the varieties and the root tips of their seedlings². The seedlings were raised by our colleagues, Messrs Crane and Lawrence, and they have described the conditions of their origin in a separate note.

Fixations were made between 2 and 20 April, 1929; this was a late season. We used medium Flemming and Navashin fluids, staining with gentian violet. Both fixatives gave very variable results with the anthers.

¹ "In Diakinese 'Pseudotetradenchromosomen' gesehen wurden, deren Verklebungen auch in die Teilungen hinein persistierten (vgl. Belar, s. 248). Die Gruppenbildung wurde zuerst als Ausdruck von intragenomatischer Affinität aufgefasst, schliesslich jedoch dem Walten des Massenwirkungsgesetzes zugeschoben."

² The apple is, of course, normally cultivated by budding or grafting on a stock, so that the roots and shoots are not genetically identical.

Root tips were cut at 4μ and anthers at 12μ (for further details compare Newton and Darlington, 1929).

III. THE CHROMOSOME COMPLEMENT IN *PYRUS*.

The following is a list of varieties and stock types examined.

	Somatic chromosome number
Allington Pippin (Text-fig. 7) ...	34
Annie Elizabeth ...	34
Beauty of Bath (Text-fig. 5) ...	34
Bramley's Seedling (Text-fig. 10) ...	51
Blenheim Orange (Text-figs. 3, 9) ...	51
Cox's Orange Pippin ¹	34
Carlisle Pippin	34
Early Victoria... ..	34
Genet Moyle (Text-fig. 8)	51
Grenadier (Text-fig. 6)	34
Irish Peach	34
Keswick Codlin	34
Kentish	34
Lane's Prince Albert (Text-fig. 4) ...	34
Lord Derby	34
Manx Codlin	34
Newton Wonder	34
Northern Spy	34
Odlins	34
Rival	34
Reinette Zuccamaglio	34
Winter Magetin	34
Worcester Pearmain (Text-fig. 1) ...	34
Old English Broadleaf Paradise (Malling Stock Type I)	34
Jaune de Metz (Malling Type IX) ...	34
Doucine (Malling Type II)	34
Nonsuch (Malling Type VI)	34

These observations, in agreement with those of Rybin (1927), show that, with an apparent basic number of 17, all cultivated varieties are orthoploid, having either thirty-four or fifty-one chromosomes. Seedlings with intermediate numbers (cf. Section V) are of feeble growth and could never usefully come into cultivation (cf. footnote, p. 146).

It will be noticed on reference to the illustrations that one type (the longest), a sub-terminally constricted chromosome, is represented four

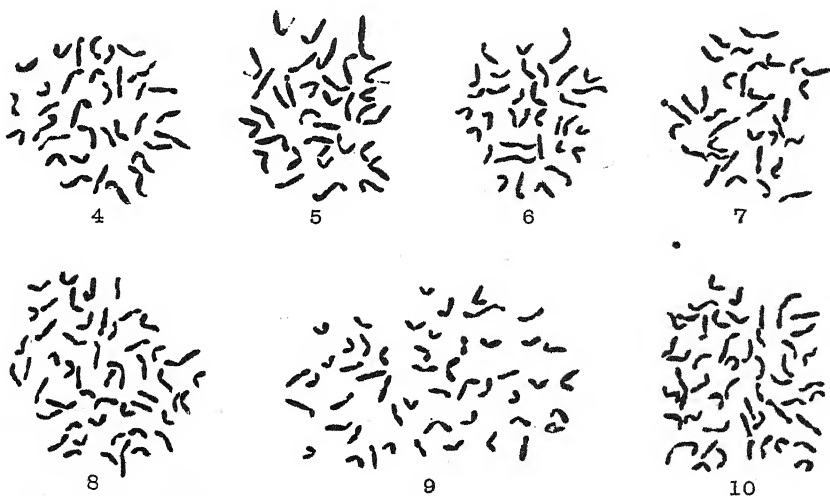
¹ The diploid Cox's Orange Pippin, like Cox's Pomona, is supposed to be a natural seedling of Ribston Pippin, a triploid (cf. Section IV). The numerical relationship shows that they are very exceptional segregates (cf. Section V).

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Text-figs. 1-3. Somatic division from root tip. ($\times 4100$.)

1. Worcester Pearmain ($2n=34$).
2. Blenheim Orange, natural seedling ($2n=38$).
3. Blenheim Orange ($2n=51$).



Text-figs. 4-10. Somatic divisions from root tip. ($\times 3000$.)

- | | |
|--------------------------------------|----------------------------------|
| 4. Lane's Prince Albert ($2n=34$). | 7. Allington Pippin ($2n=34$). |
| 5. Beauty of Bath ($2n=34$). | 8. Genet Moyle ($2n=51$). |
| 6. Grenadier ($2n=34$). | 9. Blenheim Orange ($2n=51$). |
| 10. Bramley's Seedling ($2n=51$). | |

times in the diploid and six times in the triploid. This chromosome is about 2μ long, the shortest types are less than 1μ long.

The triploid varieties evidently arise, as Rybin has shown (1927), through the functioning of a diploid (unreduced) gamete formed by a diploid individual, and not as a normal result of crossing such a form with a related tetraploid.

IV. POLLEN MOTHER-CELL DIVISIONS.

(a) General remarks.

We have examined material of the following forms:

A. Diploids:

Pyrus Ringo L.

Pyrus floribunda Kirchn.

Pyrus Malus L. vars.

Allington Pippin.

Beauty of Bath.

Cox's Orange Pippin.

Cox's Pomona.

Duchess Favourite.

Early Victoria.

Irish Peach.

Keswick Codlin.

Lane's Prince Albert.

Northern Spy.

Worcester Pearmain.

B. Triploids:

Pyrus Malus L. vars.

Baldwin¹.

Blenheim Orange.

Bramley's Seedling.

Crimson Bramley².

Ribston Pippin¹.

The more important observations in the present studies are those of polar and side views of metaphase of the first pollen mother-cell division. Either type of observation is, in our opinion, inconclusive in itself. Polar views give the clearer idea of the frequencies of different kinds of associations. They do not, however, show the physical nature of the association, for the fine connections between paired chromosomes lie for the most part axially in the spindle and are therefore invisible in polar views. In polar

¹ These varieties (not examined somatically by us) were stated by Kobel to have forty-two and forty-eight chromosomes respectively, but their appearance, behaviour and, as we shall show, meiosis are in agreement with the assumption that they are triploids and not intermediate (cf. Section V). Another variety, Reinette de Canada, which Kobel gives as having thirty-eight chromosomes from pollen mother-cell divisions, Rybin has shown to have fifty-one chromosomes from somatic divisions.

² A bud sport of Bramley's Seedling.

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views these connections can be inferred from the juxtaposition of the chromosomes. In side views they can be seen unless (as must happen very frequently) they lie obliquely to the plane of the slide. Distances, also, being more difficult to measure in the flat plate, the side view does not give so clear a general impression as the polar view. It is not surprising therefore that observations of polar views show evidence of "secondary pairing," while observations of side views show more unmistakable but less abundant evidence of "multiple association." We are inclined to take these phenomena to be the same thing seen from different points of view (cf. Lawrence, 1929; Meurman, 1929; Darlington, 1928). Whatever the value of this opinion, the conclusion to be drawn from the observations is the same, viz. that associated chromosomes are homologous. Association between genetically unrelated chromatin elements has never been proved (cf. footnote, p. 143).

Fixation was not usually so good at diakinesis as at metaphase, and deductions from the appearance of association at diakinesis are never so valuable on account of the absence of a constant relationship of the chromosomes with the spindle.

(b) "*Diploid*" forms.

The diploid forms examined all showed a high degree of regularity in the pollen mother-cell divisions; abnormalities—such as the occasional



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Text-fig. 11. Cox's Orange Pippin. Diakinesis ($2n=34$). One sexivalent, four quadrivalents, six bivalents. ($\times 3700$.)

lagging of univalents—are of sporadic occurrence. In the majority of anaphases examined seventeen bodies were found passing to each pole.

All polar views of the metaphase showed secondary association in varying degree, the multivalent associations nearly always consisting of quadrivalents and sexivalents (Text-figs. 12–14).

The maximum number of secondary associations was ten, viz. six associations between bivalents giving three sexivalents, and four such associations giving four quadrivalents. There are never more than three

sexivalents. With less than three sexivalents there are correspondingly more bivalents or quadrivalents. Thus seven is the minimum number of groups of chromosomes.

The following table indicates the frequencies of these associations in polar views taken at random from seven varieties of *P. Malus*.

TABLE I.

No. of secondary associations of bivalents:	1	2	3	4	5	6	7	8	9	10
Nos. of divisions in:—										
Northern Spy	—	—	—	3	2	5	4	2	1	1
Beauty of Bath	—	—	—	1	1	—	1	—	—	—
Keswick Codlin	—	—	—	—	—	—	—	3	1	—
Cox's Pomona	1	1	—	—	—	—	1	—	—	—
Cox's Orange Pippin	—	1	—	2	4	2	—	1	1	1
Early Victoria	—	1	—	1	1	—	—	—	—	—
Worcester Pearmain	—	—	—	1	2	1	1	1	1	—



12



13



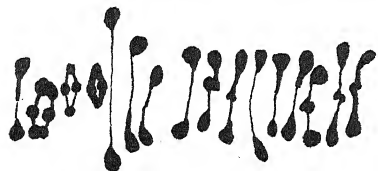
14

Text-figs. 12-14. Polar views of metaphase of the first division. ($\times 3500$.)

12. *Pyrus Ringo*. One sexivalent, five quadrivalents, four bivalents.
 13. Northern Spy. Three sexivalents, four quadrivalents (maximum association).
 14. Northern Spy. Two sexivalents, four quadrivalents, three bivalents.

As would be expected from the evidence of polar views, the commonest type of multivalent association found in side view of the metaphase is the quadrivalent (Text-figs. 15-21, complete plates). Where these lie in the plane of the slide it is possible to determine the physical nature of the association, and we were able to obtain some indication of the forms these quadrivalent associations may assume (Text-fig. 39). The different types found agreed with those illustrated by Darlington in *Prunus* (1928). Univalents were observed lying off the plate in a small proportion of cells, and occasionally a univalent and a trivalent were found in the same cell (Text-fig. 22). Sexivalents were determined less frequently in side views than in polar, but with their triangular arrangement in polar view this was only to be expected. These associations were also found in diakinesis (Text-fig. 11).

At anaphase the separation is, for the most part, regular, but fre-



15



19



16



20



17



21



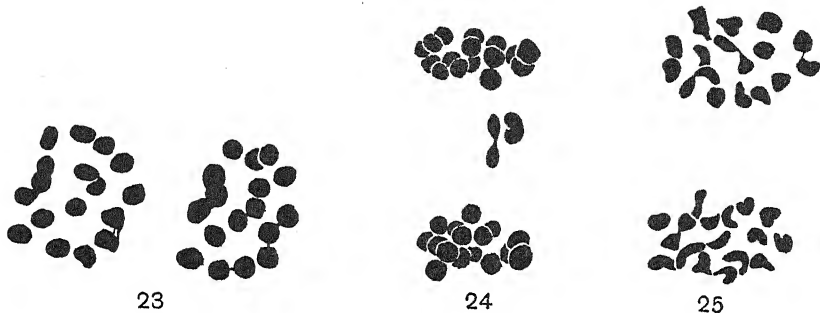
18



22

Text-figs. 15-22. Side views, metaphase of the first division in "diploid forms". ($\times 3700$.)

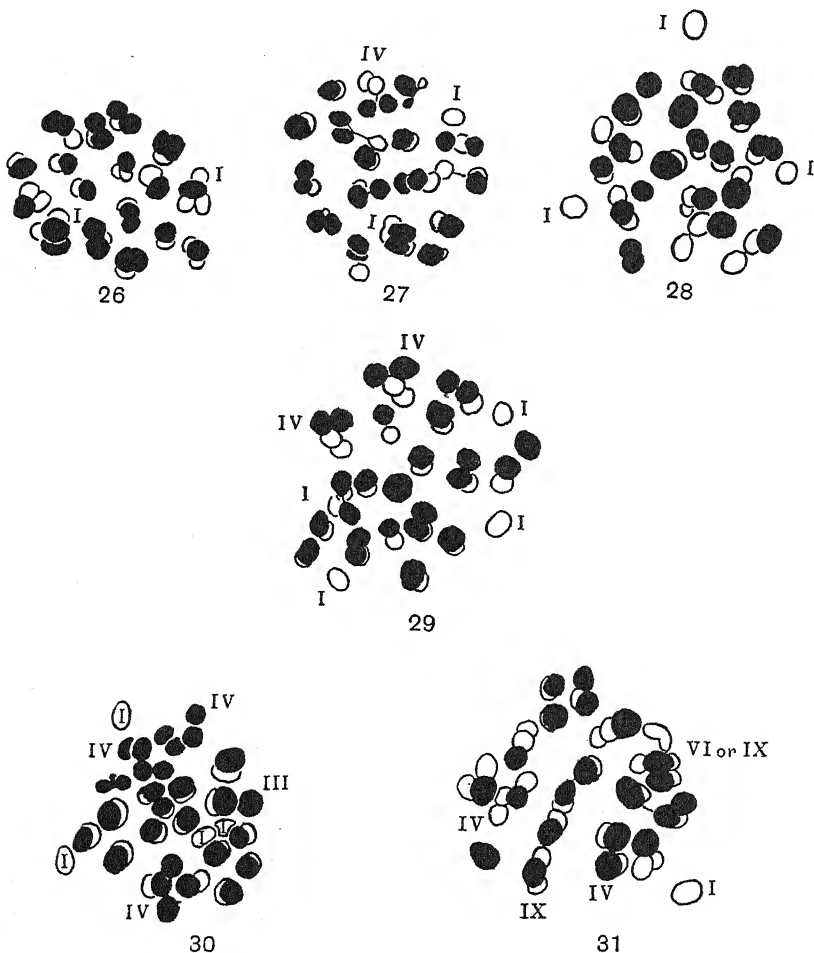
15. Cox's Orange. One quadrivalent.
16. Cox's Orange. One quadrivalent.
17. *Pyrus floribunda*. One sexivalent, two or more quadrivalents.
18. Worcester Pearmain. Two quadrivalents.
19. *Pyrus Ringo*. One quadrivalent.
20. Allington Pippin. One quadrivalent.
21. Cox's Orange. One quadrivalent.
22. Cox's Orange. One trivalent, one univalent.



Text-fig. 23. *Pyrus Ringo*, anaphase of the first division. Note persistence of multiple associations as in Text-fig. 25. ($\times 3500$.)

Text-fig. 24. Northern Spy, anaphase of the first division. Two univalents lagging. ($\times 3500$.)

Text-fig. 25. Allington Pippin. Metaphase of the second division. 17+17. ($\times 3500$.)



Text-figs. 26-31. Polar views of first metaphase in "triploid" vars. The roman numerals (I-IX) show the number of chromosomes in each association. ($\times 4500$.) See Table II.

quently a bivalent chromosome was seen late in dividing. Such bivalents probably formed part of multivalent associations and will reach the poles in time to be included in the daughter nuclei. Less frequently univalents may be found dividing on the spindle after the rest of the chromosomes have reached the poles (Text-fig. 24). These may be derived from univalents or from multivalent associations.

In polar views of the metaphase in the second division the association of chromosomes found in the first division may still persist (Text-fig. 25). This has usually been found in polyploid species and varieties where the second division rapidly follows a first division with multivalent associations.

(c) "*Triploid*" forms.

The divisions of meiosis show the special abnormalities inherent in the multivalent association of chromosomes. In one variety, Crimson Bramley, irregular distribution of chromosomes on the spindle was seen with a consequent formation of restitution nuclei and single second division plates (Rosenberg's "semi-heterotypic" divisions).

The commonest multivalent association found is the trivalent, which is of many forms (Text-fig. 38). We have identified every combination up to nine, and Table II gives an analysis of selected observations of polar views in which a fair degree of certainty was attained.

TABLE II.

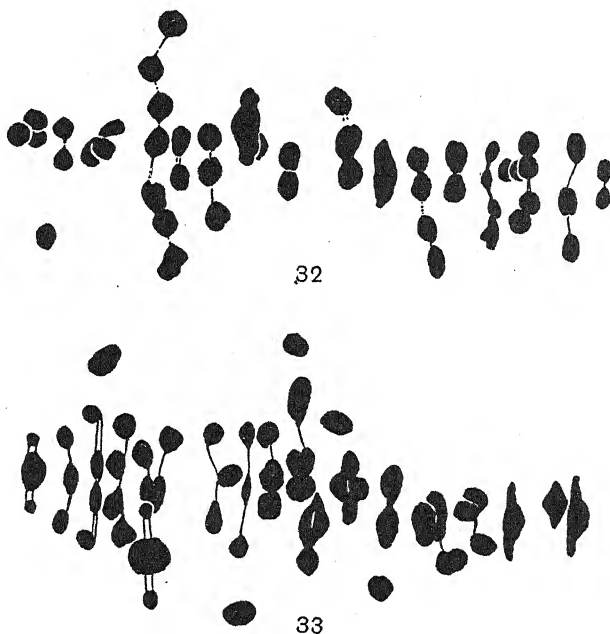
Numbers of chromosomes in various associations.

Blenheim Orange.									
A (Text-fig. 26)	2 ^I	2 ^{II}	15 ^{III}						
B (" 27)	2 ^I	7 ^{II}	9 ^{III}	1 ^{IV}					
C (" 28)	3 ^I	9 ^{II}	10 ^{III}						
D (" 29)	4 ^I	12 ^{II}	5 ^{III}	2 ^{IV}					
E (" 30)	5 ^I	14 ^{II}	2 ^{III}	3 ^{IV}					
F (" 31)	1 ^I	3 ^{II}	7 ^{III}	2 ^{IV}	1 ^{VI}	—		1 ^{IX}	
G (" 32)	2 ^I	5 ^{II}	7 ^{III}	1 ^{IV}	1 ^{VI}	1 ^{VII}			
H (" 33)	5 ^I	10 ^{II}	6 ^{III}	2 ^{IV}					
Ribston Pippin.									
K and L	3 ^I	4 ^{II}	11 ^{III}	2 ^{IV}					
Baldwin.									
M	6 ^I	6 ^{II}	10 ^{III}	1 ^{IV}					
N	2 ^I	4 ^{II}	10 ^{III}	3 ^{IV}					
O	3 ^I	12 ^{II}	8 ^{III}						

The side views illustrated (Text-figs. 32 and 33) make it clear that in some cases it will be impossible to distinguish between trivalents and bivalents in polar view (Text-figs. 26-31). Such cases number two or three in every division. It has therefore been necessary to calculate the

proportion of apparent bivalents that are really trivalents from the somatic number which is assumed in all cases to be fifty-one (see Section III).

These associations show autosyndesis within each of the three sets of the supposed triploid. The maximum association of nine corresponds to the maximum association of six in the diploid.



Text-figs. 32 and 33. Side views of metaphase of the first division, in Blenheim Orange. ($\times 4500$.) See Table II.

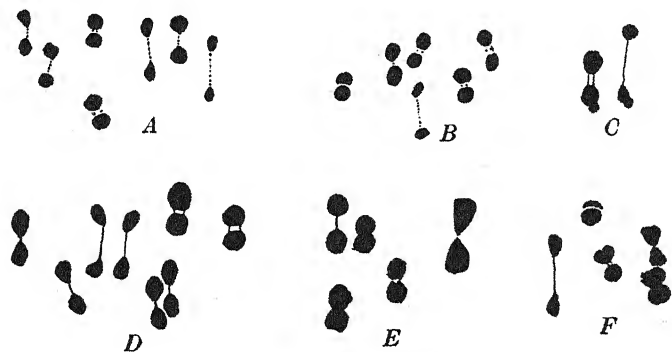
It will be noticed that rarely more than two or three univalents are found, yet as many as nine have been seen at anaphase lagging on the equator to divide after the bivalents (Text-figs. 34 and 35). It will be noticed also that where one chromosome divides before the rest it is usually, if not always, the smallest. Where this is not the case the interference of other members of a larger association may be held responsible.

The lagging of univalents which were engaged in multivalent associations, and the interference with their division by the bivalent associated, is comparable to the observations in *Hyacinthus* (Darlington, 1929 a). It was suggested that the essential difference between the division of

bivalents at meiosis, and that of chromosomes at an ordinary mitosis, is the fact that, in the former, the chromatids associated change partners at chiasmata. Thus, in their separation at anaphase, shorter lengths of chromatid have to come apart, and terminalisation of chiasmata appears

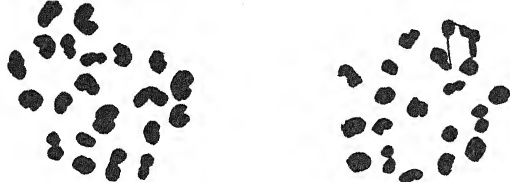


Text-fig. 34. Anaphase of the first division in Blenheim Orange. Nine univalents lagging to divide on the plate. Two fragments lying at the edge of the spindle. ($\times 4500$.)



Text-fig. 35. Groups of univalents from anaphase of the first division. *A-C*, Blenheim Orange; *D-F*, poor fixation—chromosomes swollen, from Bramley's Seedling. Note, smaller chromosomes divide first.

as an adaptation to securing ease and hence regularity of separation, for the length of chromatid to be separated is then reduced to a minimum. In this case we should expect, other things being equal, (i) that univalents would always divide after bivalents, and (ii) that long univalents



36

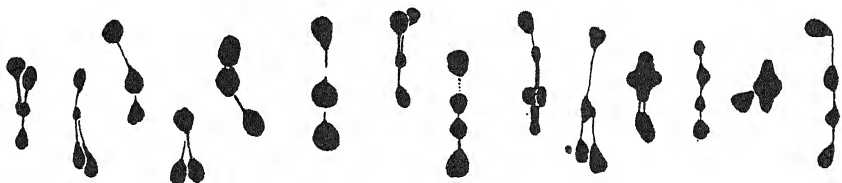


37

Text-figs. 36, 37. Metaphase of the second division in Ribston Pippin. ($\times 4500$.)
Note persistence of multiple association.

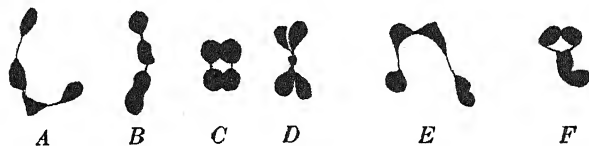
36. $29 + 1 + 21$.

37. $27 + 4 + 20$.



38

Text-fig. 38. Trivalents from metaphase of the first division in
Blenheim Orange. ($\times 4100$.)



A

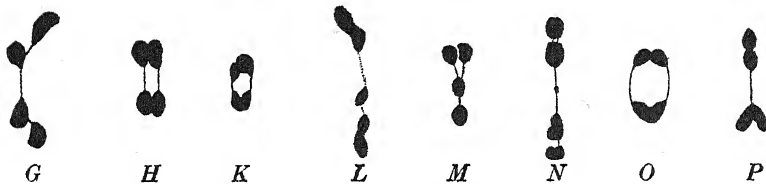
B

C

D

E

F



G

H

K

L

M

N

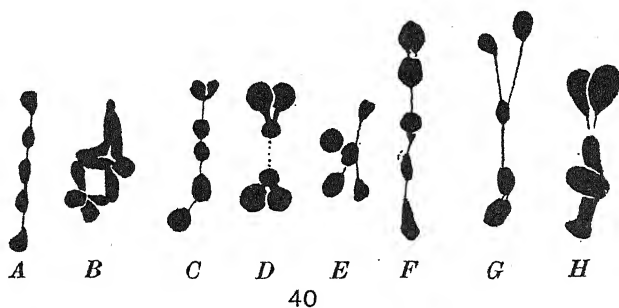
O

P

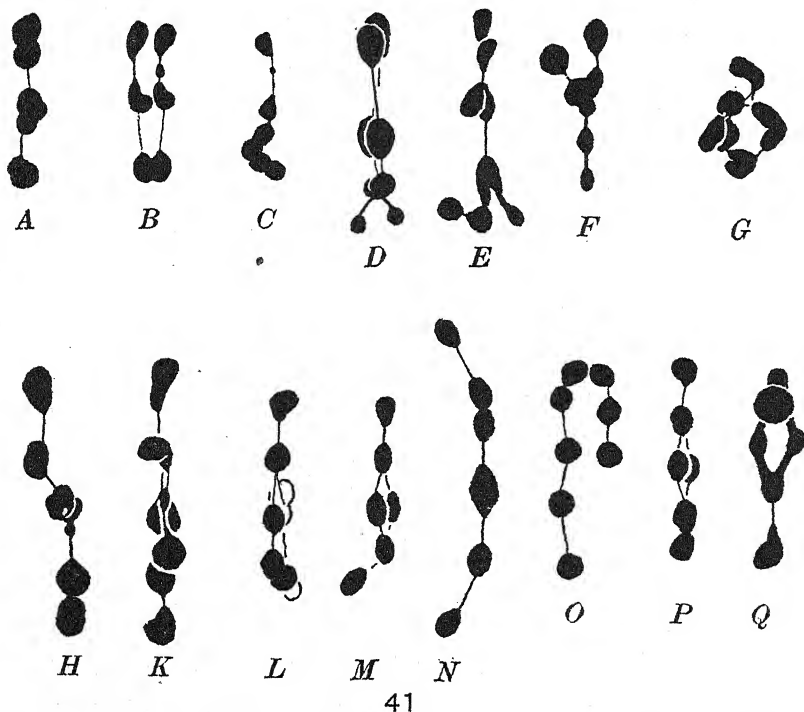
39

Text-fig. 39. Quadrivalents from first division of "diploid" forms. ($\times 4100$.) A, B and E diakinesis, the rest metaphase. A-D, *Pyrus Ringo*. E-G, Cox's Orange Pippin. H, Northern Spy. K and L, Cox's Orange Pippin. M, Allington Pippin. N, Cox's Orange Pippin. O, *Pyrus floribunda*. P, Worcester Pearmain.

should divide after short ones. The first corollary is supported by common observation, and this is perhaps evidence for the second.



Text-fig. 40. Quadrivalents from "triploid" forms. ($\times 5000$.)



Text-fig. 41. A-C, sexivalents from "diploids." ($\times 4100$.) D-Q, Bramley's Seedling and Blenheim Orange. ($\times 5700$.) A and G diakinesis, the rest metaphase of the first division. A-D, VI; E, V; F-K, VI; L, VIII; M, VI, N, VII; O, VIII, P and Q, VI.

At the second division we see the results of the multiple association, first, in a continuance of the first division relationships in those associated

chromosomes which have passed together to the same pole; and secondly, in the variable numbers in opposite plates; and thirdly, in the presence of lagging chromosomes lost in the cytoplasm (Text-figs. 36 and 37). The illustrations show numbers in second division plates from twenty to twenty-eight. These correspond with Rybin's observations of numbers in pollen grain divisions.

V. CHROMOSOME NUMBERS OF SEEDLINGS.

The chromosome numbers of seventeen seedlings were obtained from their root-tips (Table III). These seedlings (cf. Text-fig. 2) were obtained from open pollination of Bramley's Seedling, and were (cf. Crane and Lawrence) almost certainly crosses with diploid varieties.

TABLE III.

Chromosome numbers of seedlings of Bramley's Seedling.

No. of chromosomes	38	39	40	41	42	43	44	45	46	47
No. of seedlings (13)	2	1	3	4	—	1	—	—	1	1

Their appearance and the conditions of their origin are described elsewhere (Crane and Lawrence). Their chromosome numbers are exceptional amongst the offspring of triploids and, although the numbers are not highly significant, it will be noticed that such a frequency is in accordance with the assumption of secondary polyploidy. (See pp. 145-6.)

VI. SECONDARY POLYPLOIDY IN THE POMOIDEAE.

(a) Evidence.

In true-breeding diploids, where the chromatin material is physiologically differentiated, pairs of chromatin elements, or chromosomes, which segregate at random, must be identical. In diploids in which segregation of different types of gametes occurs, the pairing chromosomes must be none the less homologous, they must correspond perfectly in a physiological sense and approximately in a phylogenetic sense¹. If we admit that the homology applies to the elements only in so far as they are capable of pairing, this generalisation is probably of universal bearing². But it is enough for most purposes to regard the chromosomes as units (ignoring such minor structural changes as may occur) in

¹ The chromosomes lying at the root of all homology and analogy, the distinction becomes perhaps a little transcendental.

² In regard to this principle Yarnell (1929) has found a type of chromosome pairing in triploid *Fragaria* (with the Rosaceous set of seven), which he thinks disagrees with all previous observations in plants and animals except with those of Gates on triploid *Oenothera*. Gates, however, in the studies referred to, does not state how the chromosomes

deducing their relationships from their behaviour. This we propose to do in the case of the genus *Pyrus*, for the simple hypothesis is sufficient to explain all our observations.

In all supposedly diploid forms of *Pyrus* that we have examined there are (descriptively) two types of association of chromosomes; first, an almost invariable association of seventeen bivalents; secondly, a variable superimposed association of nine of these bivalents into three groups of three and of eight of them into four groups of four. Thus in an extreme case (such as is recorded twice, cf. Table I) we find seven groups of chromosomes instead of seventeen. Indications of the same kind are to be seen in Nebel's illustrations (1929, e.g. Fig. 26, *Malus fusca*, of which Nebel remarks "Diese Art ist orthoploid und zeigt keine weiteren Besonderheiten"). Shoemaker and Kobel, on the other hand, have apparently taken multivalent associations to be single chromosomes. In general, of course, cytologists avoid any suspicion of "clumping" like an evil spirit.

Naturally in "triploid" forms the association is more complex and more variable. Associations of nine corresponding to the six of the diploid are therefore rare, but every valency up to nine has been observed in both side and polar views of metaphase. Such high associations probably occur also in the high polyploid *Prunus laurocerasus* (Meurman, 1929). These show that the original basic number in *Pyrus* is less than half the numerical basic number of seventeen. They reinforce the direct conclusion from the diploid that there are seven types of chromosome, four of which appear four times and three, six times.

Pyrus is therefore shown by its chromosome behaviour to be functionally (with occasional exceptions) a diploid while historically it is quadruply tetrasomic and trebly hexasomic. Such a condition has been shown convincingly in only one other species¹, *Dahlia Merckii* (Lawrence, 1929).

pair or, indeed, as far as we can find, whether they pair at all. Other workers have always shown the pairing properties of the chromosomes in *Oenothera* to be specific and constant (cf. Darlington, 1929 b). The exceptional pairing is probably analogous with that found by Nishiyama (1929) in a triploid *Avena* hybrid, where more than seven pairs are sometimes formed. This phenomenon is very properly related by Nishiyama to cases of segmental interchange, and it is in this sense that we may infer an analogy with the structural hybrid *Oenothera* species, diploid and triploid.

¹ Similar numerical relationships are often due to fragmentation or perhaps fusion. *Tulipa galatica* with sixteen chromosomes probably arose by fragmentation from the common type with twelve (Newton, 1927), and similarly *Fritillaria imperialis* with twelve from a type like *F. ruthenica* with nine (Darlington, 1929 b). Before examining meiosis in *Pyrus*, we were inclined to attribute the relationship of its set to the smaller ones to similar changes in structure.

The following formulae represent the gametic or haploid constitutions of the two types for comparison.

Dahlia Merckii

AAA

BBB

CC

DD

EE

FF

GG

HH

 $(n = 18)$ *Pyrus* and related genera

AAA

BBB

CCC

DD

EE

FF

GG

 $(n = 17)$

In *Dahlia* it is possible to point to the related simple tetraploid form within the genus. Within the whole of the *Pomoideae*, however, no species occurs with the simple basic number of seven. We have to turn to *Rosa*, *Rubus*, *Geum*¹, *Fragaria* and *Potentilla*² to find direct evidence of the primary organisation. Here we are carrying inference of homology a step further than usual and we must therefore adduce evidence from other sources in support of our thesis.

A second source of evidence is derived from the seedlings of the "triploid" apple, Bramley's Seedling.

In selfing triploids or crossing them with diploids (as in *Datura*, Belling and Blakeslee, 1922; *Solanum*, Lesley, 1928; and *Crepis*, Navashin, 1929) we find a resolution in different ways of two distribution tendencies in respect of chromosome number: (i) the binomial frequency of gametes with different numbers about the half-number as a mean, found in the pollen grains of *Hyacinthus orientalis* (Belling, 1924, Darlington, 1926) and *Tradescantia brevicaulis* (Darlington, 1929 b); (ii) the elimination of a proportion of the male gametes and of zygotes with unbalanced numbers. Certain cases in *Oenothera* (cf. Gates, 1928) are exceptional; this is probably related to the exceptional organisation of the nucleus in these forms and will be specially considered elsewhere.

These seedlings of a triploid apple, few though they are, must be taken to be a sample of an exceptional population. The largest class of gametes produced should be those with twenty-five and twenty-six chromosomes, and of zygotes therefore (in the absence of elimination) with fifty-one chromosomes in a cross between two triploids and with forty-two and forty-three chromosomes in crosses with diploids. The

¹ In *Geum* (Winge, 1925) the set of seven has to be inferred in the absence of a diploid form.

² Tischler (1929).

proportion of these numbers should be increased by autosyndesis. We know, however, that selective elimination is rigorous in all stages of growth (cf. Crane and Lawrence). Yet the highest frequency is observed with forty-one chromosomes, which happens to be the sum of the presumed primary haploid number (seven) and the secondary diploid number (thirty-four). The occurrence of these types (as far as the observations go¹) is in accordance with the usual rules of inheritance in aneuploids, if we assume that the primary differentiation of the complement in *Pyrus* is into seven and not seventeen. Seedlings with forty-one chromosomes may combine the primary balance of seven with the secondary balance of seventeen.

The conclusion is strengthened from a third source by the frequencies of chromosome types at somatic mitosis, and from a fourth source in the occurrence of the primary set of seven in five important genera in other groups of the Rosaceae. Finally, evidence of the genetical complexity of "diploid" apples is given by Crane and Lawrence.

(b) *Significance.*

In *Pyrus* and *Dahlia* we have chromosome complements unbalanced, relative to the primary balanced form found in the Rosaceae and in other species of *Dahlia*. Since the new forms fulfil the requirements of existence as well as the old do or did, it would perhaps be more satisfactory to describe the original forms as having a primary balance (of seven in *Pyrus* or eight in *Dahlia*) and the new forms as having a *secondary balance* (of seventeen in *Pyrus* or eighteen in *Dahlia*).

Forms have arisen in experiment which are in various respects similar to the natural forms in these two genera. The analogy between new forms of experimental and natural origin must be considered from three points of view, viz. (i) method of origin, (ii) viability and fertility, (iii) general variation.

(i) Hexasomic tetraploids might arise simply from irregularities in the primary tetraploids (cf. *Primula kewensis*, Newton and Pellew, 1929). Doubly and trebly hexasomic tetraploids are more likely to come from the derivatives of a tetraploid-hexaploid cross. They have never been identified in experiment.

(ii) In the light of the theory of differentiation of chromatin material and Bridges' theory of genic balance (1922), there are two ways in which we may graduate the possible states of unbalance, viz. (a) by the number of times a given part of the chromatin is represented relative to the repre-

¹ They were difficult on account of the extreme weakness of the seedlings.

sensation of the rest and (b) the proportion of chromatin which is represented a given number of times in excess of the rest. In regard to the first type of graduation it is obvious that the greater disproportion should make the greater difference and this has been shown in *Datura* (Blakeslee, 1922), *Solanum* (Lesley, 1928, 1929), *Matthiola* (Frost and Mann, 1924; Lesley and Frost, 1928), *Primula kewensis* (Newton and Pellaw, 1929) and *Prunus*. In regard to the second type of graduation, it has been suggested that a single extra chromosome or $n - 1$ extra chromosomes should produce less unbalance than the reduplication of half the haploid complement (Darlington, 1929 b), and there was some evidence in favour of this view from the size of the pollen grains with different chromosome numbers. The observations on *Drosophila* and *Solanum*, however, show that the greater unbalance is less specific in its effect owing to what Bridges calls the greater internal balance of the larger parts of the complement.

The type of unbalance in *Dahlia* and *Pyrus* is such as corresponds with that in trisomic or sesquiplext (cf. de Vries, 1923) diploids. We may call it "sesquiplext" unbalance to distinguish it from a more extreme condition of "duplex" unbalance found in a tetrasomic diploid (which is the simplest unbalanced form theoretically capable of breeding true). Amongst such sesquiplext types, the *doubly* and *trebly* hexasomic combinations found in *Dahlia* and *Pyrus*, should have a more widespread effect than *simply* hexasomic forms, although, on this account, perhaps, a less drastic effect on vigour and fertility.

We believe, therefore, that the two sets of observations are compatible. The secondary forms are the isolated successes, not merely from the 126 ($2^n - 2$) sesquiplext combinations possible in any given individual in *Pyrus* (with a set of seven) and the 254 such combinations in *Dahlia* (with a set of eight), but from a much greater number of natural experiments in unbalanced forms that have been tested in the Rosaceae over a vast period of time. Since evolution proceeds largely, if not entirely, by changes in the balance of the hereditary materials¹, it is plausible that this method of change, the extreme of discontinuity, will in one case out of a very large number yield (at least with later selection) a product as vigorous and as fertile as its antecedents².

(iii) The same experimental evidence (and also that in *Oenothera*, cf.

¹ This conclusion is emphasised by the numerous observations that have recently been made on structural differences between the chromosomes of related species and varieties (cf. Darlington, 1929 b).

² It must be remembered that the difference between balance and unbalance is merely the difference between a system that has been tested by natural selection and one that has not. The difference therefore depends upon chances.

Gates, 1928; *Crepis*, Navashin, 1929; and *Drosophila*) shows beyond doubt that, whatever the success in viability and fertility of a secondarily balanced form, it will be very different from its progenitors in general properties.

The change to a new, relatively unbalanced, complement should mean a definite evolutionary step of a kind incompatible with the maintenance of the primary series of seven, unless by the gradual accumulation of structural changes. This can be tested, and in *Dahlia* it is significant that Lawrence (1929) remarks that: "*Dahlia Merckii* is a very distinct species and the only one I have seen with fertile ray florets." We think it plausible that *Dahlia Merckii* owes some of its distinctive properties to its unbalance as compared with its relations, and this is equally likely with the *Pyrus* group. In both cases the morphological expression of the secondary balance must be different from that of the primary balance¹.

Related species with apparently unrelated chromosome numbers are of widespread occurrence, and may be derived from one another by processes associated with structural change or unbalance or both. A distinction between the two methods can be seen when simple polyploidy is contrasted with the effects of unbalance. Polyploid forms may be found, such as *Silene ciliata* (Blackburn, 1928) and *Prunus domestica* (Darlington 1928), which are scarcely distinguished systematically from their lower multiple relatives. While undoubtedly a multiplication of chromosome number provides a new vehicle for the origin of variations and their expression, and provides the possibility of fertility for sterile hybrids, it does not replace unbalance and structural change as original means of variation. Both changes, however, are facilitated by polyploidy, and particularly the cruder change of numerical unbalance².

Cytological literature is deficient in evidence, from which we can conclude the relative importance and the degree of interdependence of these two primary types of variation³. Approach to the problem on the

¹ Further study will, we hope, throw some light on the detailed implications of this view.

² Physiologically both are "unbalance." The distinction is purely mechanical.

³ For example: Randolph (1928) hesitates to arrive at any definite conclusion as to the "fundamental nature of the extra chromosomes" in *Zea*. J. Clausen (1928) depends on number of chromosomes alone for evidence of relationship in *Viola*, and seems to us to ignore evidence of secondary pairing in polyploid forms. Compare, for example, Clausen's fig. 14 (1929) with his remarks on *Viola glabella*. Kazao (1928) has found numbers in *Iris* which suggest the type of change here assumed in *Pyrus*. More recently, Håkansson (1929) has found forms of *Scirpus* in a similar numerical relationship to that assumed in *Pyrus*. He attributes the increase in number to fragmentation. In another study he shows the probable secondary association in *Salix caprea* ($n=19$), but does not comment upon it. Such instances might be multiplied.

lines suggested in the present essay will, we believe, gradually remove this difficulty.

VII. SUMMARY.

1. The basic chromosome number in *Pyrus* is seventeen. Cultivated varieties are all orthoploid. Aneuploid seedlings are poor and abnormal.

2. The somatic chromosomes in "diploid" *Pyrus* have four representatives of a long type, in "triploid," six.

3. Multiple association occurs amongst the chromosomes of "diploid" *Pyrus* giving, in extreme cases, seven groups; four quadrivalents and three sexivalents (Table I).

4. In "triploid" varieties of *P. Malus* associations of four, five, six, seven, eight and nine chromosomes have been observed, although trivalents are usually formed (Table II). This means that autosyndesis takes place within each of the three supposed haploid complements.

5. Instead of giving a binomial frequency or the elimination of intermediate numbers, natural seedlings of "triploid" apples most frequently have numbers of chromosomes approximately to $2n + 7$ (Table III).

6. Thus the pairing, morphology, and breeding results show, directly or indirectly, that the thirty-four chromosomes in the "diploid" *Pyrus* are of seven types, of which four are represented four times and three are represented six times. Such forms may be described as trebly hexasomic tetraploids (v. diagram, p. 145).

7. The number seventeen is therefore a secondary (unbalanced) basic number, and the derived series of polyploids ($2n = 34, 51, 68$) are *secondary polyploids*.

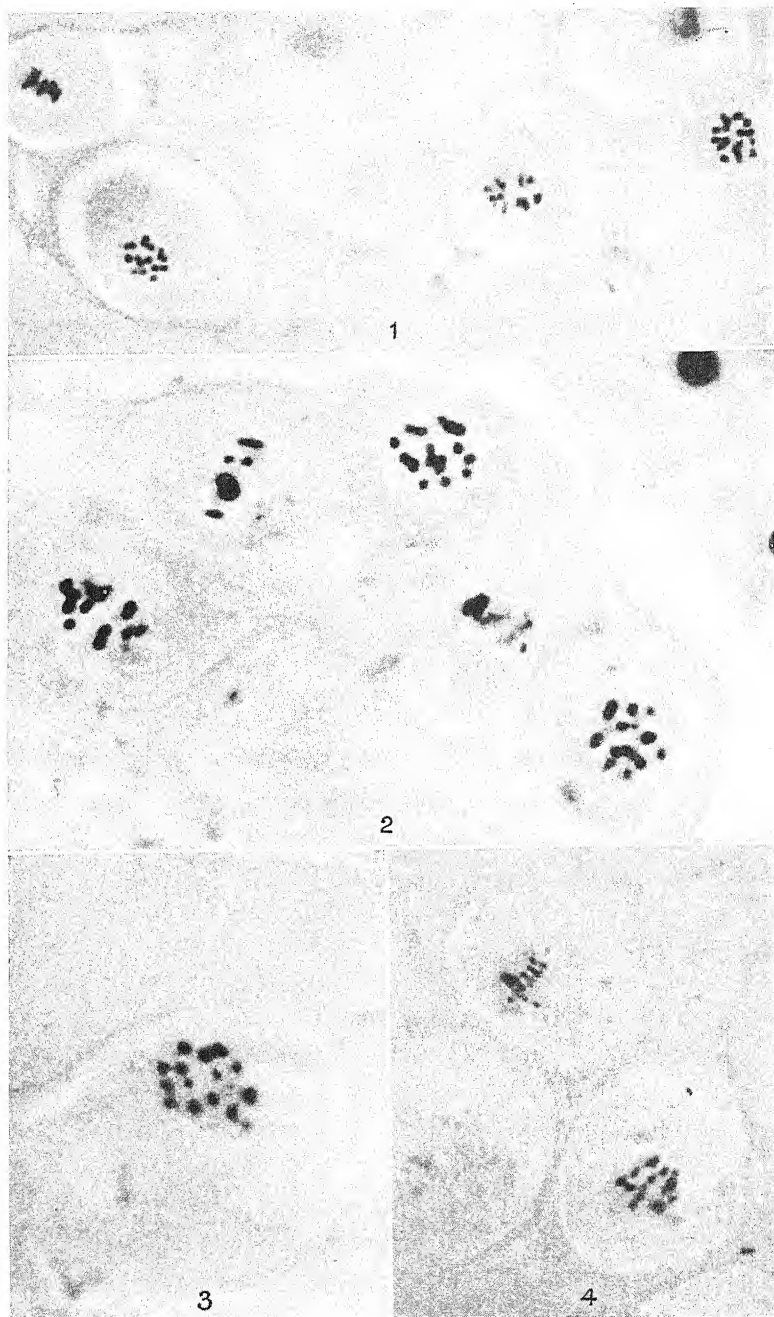
8. The establishment of a secondary basic number must mean (on the analogy of all experimental observations) a definite evolutionary step. It is therefore plausible that the *Pyrus* group owe their special morphological characters (e.g. the pome type of fruit) to this reorganisation of the nucleus. The work is being continued with this consideration in view.

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DESCRIPTION OF PLATE V

Figs. 1-4. Microphotographs of the first pollen mother-cell division in "diploid" and "triploid" apples showing multivalent associations.

1. Northern Spy. ($\times 1900$.) Left-hand division drawn in Text-fig. 14.
2. Northern Spy. One possible sexivalent, five quadrivalents, four bivalents. ($\times 1200$.)
3. Blenheim Orange. ($\times 2500$.) Same as Text-fig. 26.
4. Blenheim Orange. ($\times 1500$.) Same as Text-fig. 31. In the top division note multivalents lying across the plate.



FERTILITY AND VIGOUR OF APPLES IN RELATION TO CHROMOSOME NUMBER.

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INTRODUCTION.

EXPERIMENTS relating to sterility and incompatibility in plums, cherries and apples have been carried out at this Institution since 1911. Reports on the results obtained have appeared from time to time, and in the last publication (Crane and Lawrence, 1929) the results of the investigations from 1911 to 1928 were briefly summarised.

As the experiments progressed it became evident that among cultivated apples sterility and incompatibility differed in several respects from the similar phenomena in plums and cherries. The salient differences were: (1) as far as our investigations go, incompatibility occurs in varying degree, but is rarely, if ever, completely expressed in apples, as it is in plums and cherries¹; (2) as a rule the degree of generational sterility is much higher in apples—especially in certain varieties—than in cultivated varieties of plums and cherries; and (3) in certain crosses not only were few seeds formed, but the seedlings which germinated were extremely weak and remained feeble throughout their growth.

¹ About 50 distinct varieties of apples have been used, and out of a total of 243 cross-pollinations, involving over 23,000 flowers, only five entirely failed to give fruits. As comparatively few flowers were used in these five crosses too much importance should not be attached to them and repetition is desirable.

By way of comparison, all the sweet cherries (= varieties of *Prunus avium*) we have used are self-incompatible, and out of 36 varieties 20 form six incompatible groups within which all self- and cross-pollinations fail. In plums, *Prunus domestica*, the behaviour of incompatibility is more complex, but complete self-compatibility and self-incompatibility is common, and several examples of cross-incompatibility have been established. The above cherries are approximately diploid and the plums hexaploid ($2n=48$).

POLYPLOIDY IN RELATION TO FERTILITY.

Until comparatively recently these experiments were carried out without any knowledge of the chromosome complement of the various forms used, but during the past few years cytological investigations have shown that among our cultivated fruits polyploidy is common.

In general odd multiple polyploids are relatively infertile, and apparently the only known exception to this is in the hyacinth, where triploid forms set seed comparatively freely. Where seed and fruit development are closely associated, as in *Prunus* and *Rubus*, triploid and other odd multiple forms are relatively unproductive. When such forms do appear in cultivation, as for example in *Rubus*, their fertility and productivity is lower than that of even numbered and balanced polyploid varieties. In a recent paper (Crane and Darlington, 1927) an apparent exception was described, viz. the Himalaya berry, but this has been re-examined and found to be tetraploid ($2n = 28$). In *Prunus* the only triploids known to occur are ornamental varieties grown for their flowers.

In both *Prunus* and *Rubus* development of the bi- or uni-ovular drupes is very largely dependent on the development of the seed. In *Pyrus*, however, the greater number of seeds per fruit and the much lower proportion of fruit to flower required to give a crop enables the triploid to become fruitful enough to be of economic importance. In apples a single seed is often sufficient for the development of the fruit, and even this seed may be imperfect. This approaches parthenocarpy and renders fruit production still less dependent on the formation of seeds. In some varieties of apples entirely seedless fruits are not uncommon.

"TRIPLOIDY" IN APPLES.

Rybin (1926) first observed triploidy in apples, finding Reinette du Canada, an established variety, to be triploid ($2n = 51$). Kobel (1927) reported 13 of the varieties he examined to be diploids ($n = 17$) and 15 varieties to have intermediate numbers (see Table I). In a previous paper we assumed several of his varieties with intermediate numbers to be triploids, viz. Warner's King, Ribston Pippin, Gravenstein and Baldwin. In a recent paper Nebel (1929) has shown that Ribston Pippin, Gravenstein and Baldwin are triploids. He particularly investigated the Gravenstein group of apples (apparently a group of eight clonal variations) and found them all to be triploid. Bramley's Seedling and its bud-sport, Crimson Bramley, are also triploids. In our publication

(1929) we gave an account of preliminary observations and showed that Blenheim Orange, Bramley's Seedling and Genet Moyle were triploid varieties. If we take Kobel's varieties with intermediate numbers to indicate triploidy, and include the clonal variations of Gravenstein and Bramley's Seedling, a total of 24 varieties has been found to be triploid and about 48 varieties to be diploid. It is therefore evident that among cultivated apples triploidy is common.

Darlington and Moffett (1930), in the paper which this article accompanies, show that the chromosome complement in apples is complex, and have concluded that the forms with $2n = 34$, generally regarded as diploids, are secondary polyploids, being hexasomic in respect of three chromosomes and tetrasomic in four. Thus the varieties we refer to as "triploids" are partly hexasomic and partly nonasomic.

TABLE I.

Variety	Chromosome numbers ($2n$)				% pollen germination		
	Rybin	Kobel	Nebel	Darlington and Moffett	Kobel	Kvaale	Florin
Baldwin	—	48-49	51	51	11.0	12.3	0-30
Belle de Boskoop	—	ca. 46	51	—	13.0	—	0-30
Blenheim Orange	—	—	51	51	—	—	0-30
Bohnapfel	—	46-49	—	—	10.0	—	—
Bramley's Seedling	—	—	—	51	—	20.9	0-30
Crimson Bramley	—	—	—	51	—	—	—
Damason Reinette	—	45-47	—	—	23.0	—	—
Genet Moyle	—	—	—	51	—	—	—
Gravenstein	—	45-46	51	—	7.0	13.0	0-30
Gravenstein (7 clonal varieties)	—	—	51	—	—	—	—
Harbert's Reinette	—	45	—	—	16.0	—	—
Jacques Lebel	—	49-51	—	—	13.0	—	—
Reinette du Canada	51	38-40	51	—	4.0	—	0-30
Ribston Pippin	—	42	51	51	—	21.4	0-30
Roter Eiseraffel	—	47	—	—	—	—	—
Stäfner Rosenapfel	—	48-49	—	—	25.0	—	0-30
Warner's King	—	42	—	—	27.0	14.8	0-30
Winter Zitronenapfel	—	48-49	—	—	21.0	—	0-30
Lane's Prince Albert (1)	—	—	—	34	—	57.6	70
Lane's Prince Albert (2)	—	—	51	—	—	—	—

In Table I are given the chromosome numbers of the varieties under discussion as found by Rybin, Kobel, Nebel, and Darlington and Moffett, and the percentage of pollen germination as found by Kobel (1927), Kvaale (1926) and Florin (1926). At the bottom of the table is the variety, Lane's Prince Albert, which was reported by us (1929) to be a diploid ($2n = 34$), and by Nebel (1929) as a triploid ($2n = 51$). We have no reason to doubt either our material or observations. Nebel, however,

states that our breeding results agree with the conception of Lane's as a triploid, but the argument he advances is insufficient and ignores the fact that it is only in certain crosses with Lane's that relatively poor results are obtained. Presumably he refers to the results presented in Table IX of our paper, viz. Cox's Orange Pippin (a diploid) \times Lane's Prince Albert and its reciprocal, in which only 2.7 and 1.6 seeds per fruit were obtained from 26 and 22 fruits respectively. Among the diploid varieties of apples varying degrees of generational sterility occur, nor can the possibility of zygotic lethals be precluded. We believe, however, that the few seeds obtained in the above and similar examples where diploid \times diploid gave poor results are mainly due to degrees of incompatibility. But the "poor" seed results obtained when a triploid is involved in crosses are mainly the expression of its high degree of generational sterility, and no appreciably better results will be secured by crossing it with any other variety. On the other hand, lack of incompatibility is the expression of genetic differentiation, and as such will vary according to the particular parents used. For example, Lane's \times Cox's gave only 1.6 and Cox's \times Lane's 2.7 good seeds per fruit. Royal Jubilee \times Lane's, however, gave 5.4 good seeds per fruit, a much higher proportion than a diploid-triploid cross has ever given in our experiments. It will be noted in Table II that the vigour of the seedlings from the Lane's-Cox's crosses further confirms the diploid constitution of Lane's, and that the pollen tests of Florin and Kvaale are also in agreement.

Kobel, Kvaale and Florin made extensive pollen-germination tests on apples, and from their work it appears to be possible to discriminate between diploid and triploid varieties by the percentage of pollen germination. Kobel and Kvaale give the actual percentage of pollen which germinated, but Florin divides his results into three classes: (1) "poor," with an average pollen-germination not above 30 per cent.; (2) "medium," 30-70 per cent.; (3) "good," at least 70 per cent.

In Table I we have tabulated their results, and it will be seen that all the triploids used by Florin in his germination tests are in his "poor" class (less than 30 per cent. good) and those examined by Kobel and Kvaale range from 4 to 27 per cent. By comparison the varieties at present known to be diploid, with one exception¹, range from 50 to 97 per cent.

Although there is considerable variation in the proportion of good pollen among the known diploids, the worst diploid has a much higher proportion than the best triploid.

¹ The exception is Allington Pippin, a diploid variety included in Florin's "poor" germinating class.

The variations in generational sterility among diploids may be due to two causes: (i) segregation of dissimilar chromosomes normally pairing; (ii) irregularities resulting from the formation of quadrivalents, *i.e.* from an insufficient differentiation of the usually non-pairing homologues in a polyploid (cf. Darlington and Moffett, Table II). The low fertility of Cox's Orange, a diploid, alleged to be a seedling from Ribston Pippin, a triploid, is probably due to the second of these causes. In this connection it is significant that in certain crosses made with Cox's Orange as one parent the progeny have been distinctly more vigorous and fertile than Cox's Orange.

VIGOUR IN RELATION TO CHROMOSOME NUMBER

In Table II germination results and growth measurements are given (*a*) of families derived from diploid \times diploid varieties, and (*b*) of families where one of the parents is a triploid. The measurements were taken in November 1929, after the 1927 families had completed two seasons' and the 1928 families one season's growth. The seedlings were grown under uniform conditions without pruning or any other interference. The difference between the average height for the diploid \times diploid and the diploid \times triploid families is very large, and the vigour of the former contrasts strikingly with the feeble growth of the diploid-triploid offspring.

A number of the seedlings of the family 6/28 (natural seedlings from

TABLE II.

Fam. No.	Parentage	Flowers pollinated	Fruits matured	Apparently good seed	Good seeds per fruit	No. seeds germinated	No. seedlings surviving	Average height of seedlings in inches
Diploid \times Diploid								
1/27	Lane's Prince Albert \times Cox's Orange Pippin	87	10	27	2.7	26	25	30.2
3/27	Cox's Orange Pippin \times Lane's Prince Albert	110	8	10	1.2	8	7	24.1
9/27	Cox's Orange Pippin \times Peasgood's Nonsuch	89	10	28	2.8	28	18	18.2
Diploid \times Triploid								
2/27	Lane's Prince Albert \times Blenheim Orange	18	3	9	3.0	8	3	3.5
4/27	Cox's Orange Pippin \times Blenheim Orange	160	12	14	1.1	9	4	6.1
5/27	Peasgood's Nonsuch \times Blenheim Orange	42	2	4	2.0	2	2	9.1
Triploid								
7/27	Blenheim Orange selfed	218	5	11	2.2	3	1	7.3
Diploid \times Diploid								
3/28	Northern Spy \times Old English Broadleaf	117	10	84	8.4	82	78	12.0
1/28	Jaune de Metz \times Northern Spy	—	—	—	—	—	22	16.2
Diploid \times Triploid								
4/28	Cox's Orange Pippin \times Blenheim Orange	36	3	5	1.6	2	1	2.0
6/28	Lane's Prince Albert \times Blenheim Orange	109	4	2	0.5	2	1	4.5
Triploid								
7/28	Bramley's Seedling—open pollination	—	60	135	2.0	94	74	3.0

Bramley's Seedling) have been examined and all were found to be aneuploids (see Darlington and Moffett, Table III).

It is significant that all the established varieties of apples examined by Rybin, Nebel and ourselves were diploids or triploids, and that re-examination of five of the varieties reported by Kobel to be aneuploids showed them to be triploids.

Further examination of the other varieties reported by Kobel to be aneuploids is desirable, but on present knowledge it seems probable that aneuploid forms do not occur among cultivated apples, and that it would be exceptional to obtain fertile and vigorous offspring by breeding triploids either *inter se* or with diploids. It may be remarked that triploid varieties are invariably vigorous.

Reference to lack of vigour in seedlings is common in the literature relating to breeding experiments with apples. Wellington (1924) reports that 27 trees of Baldwin selfed were planted in 1909 and that all but one died, and when 11 years old the one that survived was a small and weak tree. Wellington also used the variety Gravenstein in crosses, sometimes as a male and sometimes as a female parent, and from the weakness of many of the offspring he concluded that Gravenstein carried one or more determiners for weak growth. Lantz (1925) found that the progeny from certain crosses were decidedly less vigorous than those of others. He also states that Crandall (1924), from work on 148 forms of *Malus* involving 22,619 pollinations, secured 1486 fruits or 6.5 per cent. A total of 2840 selfed seeds gave 703 seedlings, 219 of which survived. Dickson (1928) also refers to the variability of vigour in apple seedlings.

In our own breeding work, most of the seedlings raised from selfing apples, whether $2n$ or $3n$ varieties, have usually been weak and many have died. Two varieties, however, which as yet have not been cytologically examined, viz. Antonovka and Rev. W. Wilks, have given quite vigorous offspring when selfed. The selfed seedlings from Antonovka are as large and vigorous as any we have raised from crosses. Approximately a quarter of the selfed seedlings from Rev. W. Wilks were albinos and died. The internodes of the growth of Rev. W. Wilks are comparatively short, and its viable seedlings are in general dwarf, but their leaves are large and the trees are by no means weak.

The chromosome complement of most of the varieties used by the above workers is not known, but it is interesting to note that the varieties Baldwin and Gravenstein used by Wellington are triploids, and consequently the extreme feebleness of their offspring is probably due to an unbalanced aneuploid chromosome constitution.

It is evident that cytological examination is an essential preliminary to any critical investigation relating to sterility or breeding work with apples. The present cytological observations of Darlington and Moffett, in conjunction with our genetical observations, however, make it possible for us to say fairly definitely from fertility and progeny whether a particular apple is $2n$, $3n$ or aneuploid.

STERILITY AND INCOMPATIBILITY.

Fruitfulness in cultivated fruits is associated with:

- (1) Generational fertility.
- (2) Compatibility.
- (3) Morphological fertility, *i.e.* no suppression of sex organs.
- (4) Parthenocarpy.
- (5) Number of seeds per ovary¹.
- (6) Variation in the proportion of fruit to flowers required to give an economic yield (this is usually correlated with fruit size).

TABLE III.

	% of fruits necessary for good crop	% of embryos necessary for good crop
<i>Rubus</i>		
1 embryo per ovule	50-100	50-100
Many ovules per fruit		
<i>Prunus</i>		
1 embryo per ovule	19-32	*10-20
2 ovules per fruit		
<i>Pyrus</i>		
1 embryo per ovule	5-10	0-1
10 ovules per fruit		

* The proportion of fruits with double embryos in *Prunus* varies with the maternal variety. This is not incompatible with the view assumed that the development of the embryos is independent because, for example, where only 10 per cent. of the ovules develop, the 19 per cent. of fruits formed will have double embryos in the proportion of 1 : 18, while, with 99 per cent. of the embryos developing, double embryos will be in the proportion 9 : 2 (81 : 18).

In all cases initial embryos are referred to because, as we have previously pointed out (Crane, 1926), although fertilisation is always essential for fruit development in *Prunus*, the fruits may reach maturity following an embryonic breakdown. This is more common in cherries than in plums.

An approximate estimation of the influence of (4), (5) and (6) above on fruit production is tentatively presented in Table III. It will be seen that those apple varieties which are relatively infertile may yet maintain

¹ Many varieties of apples set seed freely; others set few seeds.

their economic value (1) because of the small number of fruits required to give a satisfactory yield (often 5 per cent. is sufficient), and (2) because fertilisation apparently supplies the requisite initial stimulus to fruit development, and a subsequent embryonic breakdown does not necessarily arrest the growth of the fruit. Thus analysis of over 23,000 pollinations involving 243 different crosses ($182\ 2n \times 2n$; $55\ 3n \times 2n$; and

TABLE IV.

Parentage (1)	Flowers	Fruits matured	Seeds			Good seeds per fruit
			Appa- rently good	Shriv- elled	Empty testas	
Cox's Orange Pippin ($2n=34$) selfed	1950	13	10	10	38	0.7
Lane's Prince Albert ($2n=34$) selfed	481	9	7	0	7	0.7
Charles Ross selfed	35	1	1	0	0	1.0
Ellison's Orange selfed	229	4	9	0	2	2.2
Mother selfed	284	2	5	0	1	2.5
(2)						
Cox's Orange Pippin ($2n=34$) \times Ellison's Orange	336	15	6	42	52	0.4
Lane's Prince Albert ($2n=34$) \times McIntosh Red	23	2	1	0	20	0.5
Jaune de Metz ($2n=34$) \times Northern Spy ($2n=34$)	307	11	13	0	1	1.1
Cox's Orange Pippin ($2n=34$) \times McIntosh Red	326	21	38	32	75	1.8
Lane's Prince Albert ($2n=34$) \times Ellison's Orange	32	1	2	1	6	2.0
Charles Ross \times Ellison's Orange	23	3	8	5	14	2.6
Lane's Prince Albert ($2n=34$) \times Mother	20	4	13	0	40	3.2
Lane's Prince Albert ($2n=34$) \times Cox's Orange Pippin ($2n=34$)	70	5	18	2	27	3.6
Margil \times Cox's Orange Pippin ($2n=34$)	50	7	49	6	0	7.0
Northern Spy ($2n=34$) \times Malling Type VII	29	1	7	2	0	7.0
Ellison's Orange \times Cox's Orange Pippin ($2n=34$)	121	1	9	0	0	9.0
(3)						
Blenheim Orange ($2n=51$) selfed	339	8	12	1	12	1.5
Ribston Pippin ($2n=51$) selfed	123	8	20	6	13	2.5
(4)						
Lane's Prince Albert ($2n=34$) \times Blenheim Orange ($2n=51$)	38	2	0	1	11	0.0
Cox's Orange Pippin ($2n=34$) \times Blenheim Orange ($2n=51$)	155	8	0	5	47	0.0
Cox's Orange Pippin ($2n=34$) \times Ribston Pippin ($2n=51$)	124	9	1	10	48	0.1
Lane's Prince Albert ($2n=34$) \times Crimson Bramley ($2n=51$)	57	4	3	7	12	0.7
(5)						
Blenheim Orange ($2n=51$) \times Ellison's Orange	24	2	1	1	10	0.5
Crimson Bramley ($2n=51$) \times Cox's Orange Pippin ($2n=34$)	10	1	1	1	6	1.0
Blenheim Orange ($2n=51$) \times Cox's Orange Pippin ($2n=34$)	107	8	11	17	26	1.3
Blenheim Orange ($2n=51$) \times McIntosh Red	79	7	15	11	15	2.0
Blenheim Orange ($2n=51$) \times Mother	59	1	2	2	2	2.0
(6)						
Ribston Pippin ($2n=51$) \times Blenheim Orange ($2n=51$)	12	1	0	2	2	0.0
Blenheim Orange ($2n=51$) \times Ribston Pippin ($2n=51$)	35	3	3	3	5	1.0
Blenheim Orange ($2n=51$) \times Crimson Bramley ($2n=51$)	72	1	2	0	0	2.0
Crimson Bramley ($2n=51$) \times Blenheim Orange ($2n=51$)	51	1	2	2	4	2.0

$5\ 3n \times 3n$) shows that triploid-diploid crosses are slightly more fertile than diploid-diploid, giving 6.8 and 6.1 per cent. of fruit to flowers respectively. The seed content of the fruits of these two series of crosses is, however, markedly different, and reveals a higher degree of generational sterility operating in triploid-diploid combinations.

In the past the measure of fertility has often been taken from the proportion of fruit set to flowers pollinated, but it is evident that this is merely an estimation of fruitfulness, and that examination of the seeds provides a better measure of fertility. Indeed, as is shown in Table II, the best criterion of fertility is the vigour of the progeny of the plant.

In Table IV will be found the results we obtained in 1929 from self- and cross-pollinations. Where known, the chromosome numbers follow the names of the varieties, which as far as possible are arranged as follows: (1) diploids selfed, (2) diploids \times diploids, (3) triploids selfed, (4) diploids \times triploids, (5) triploids \times diploids, (6) triploids \times triploids. It is apparent that although a low degree of fertility occurs in certain diploid-diploid crosses, none of the combinations involving triploids has given a result commensurate with the best of the diploid crosses. As we have previously pointed out, although a good deal of generational sterility occurs even among diploids, and the possibility of zygotic lethals cannot be precluded, there is but little doubt that the variation within the diploid crosses is often due to degrees of incompatibility.

Since the triploid varieties of apples have a range of only 4 to 27 per cent. good pollen, whereas the diploids range from 50 to 97 per cent., it is obvious that, if we neglect incompatibility, the diploids would be more effective as pollinisers in the field for other varieties than would the triploids. As is shown above, however, the triploid combinations have in our experiments given even slightly better results in the production of fruits than the diploids. Since incompatibility is due to lack of genetic differentiation, the good results obtained from the triploids are probably due to a greater variety in the gametic output of triploid than of diploid varieties, thereby providing a greater chance of compatible combinations. The high degree of generational sterility of the triploids is expressed by the formation of imperfect seeds and weak offspring, rather than by failure to form fruits.

In the approximately diploid sweet cherry *P. avium* incompatibility is clearly expressed, self-incompatibility is the rule and cross-incompatibility common. In the tetraploid sour cherries, *P. cerasus* and the Dukes, tetraploidy appears to have removed the bar to self-compatibility as degrees of self-compatibility occur. In the hexaploid plums, *P. domestica*, complete self- and cross-compatibility, complete self- and cross-incompatibility and degrees of self- and cross-incompatibility occur. In apples degrees of incompatibility are common even in the so-called diploid varieties; this may be attributed to their secondary polyploid complement, which involves a polysomic condition of the incompatibility

factors. Every chromosome and hence its factors may be represented two or three times in the gametophyte, which provides a basis for greater variation in the number of possible combinations of a given factor.

Admitting that the "diploid" apples are secondary polyploids, it is worthy of note that degrees of compatibility are expressed in the yield of polyploids only, *viz.* the sour cherries, the domestic plums and apples.

SUMMARY.

Odd multiple polyploids are relatively infertile, consequently in fruits such as *Rubus* and *Prunus*, where seed and fruit development are closely associated, triploid and other odd multiple forms are relatively unproductive. Triploid apples however are productive, *e.g.* Bramley's Seedling, a triploid, is probably more widely cultivated in this country than any other apple.

In apples a very low proportion of fruit to flowers is sufficient to give a yield. The apple has ten embryos, and often a single seed is sufficient for the development of a fruit, and even this seed may be imperfect. This approaches parthenocarpy and renders fruit production still less dependent on the formation of seeds. Fruitfulness in apples may therefore be maintained in spite of a high degree of generational sterility.

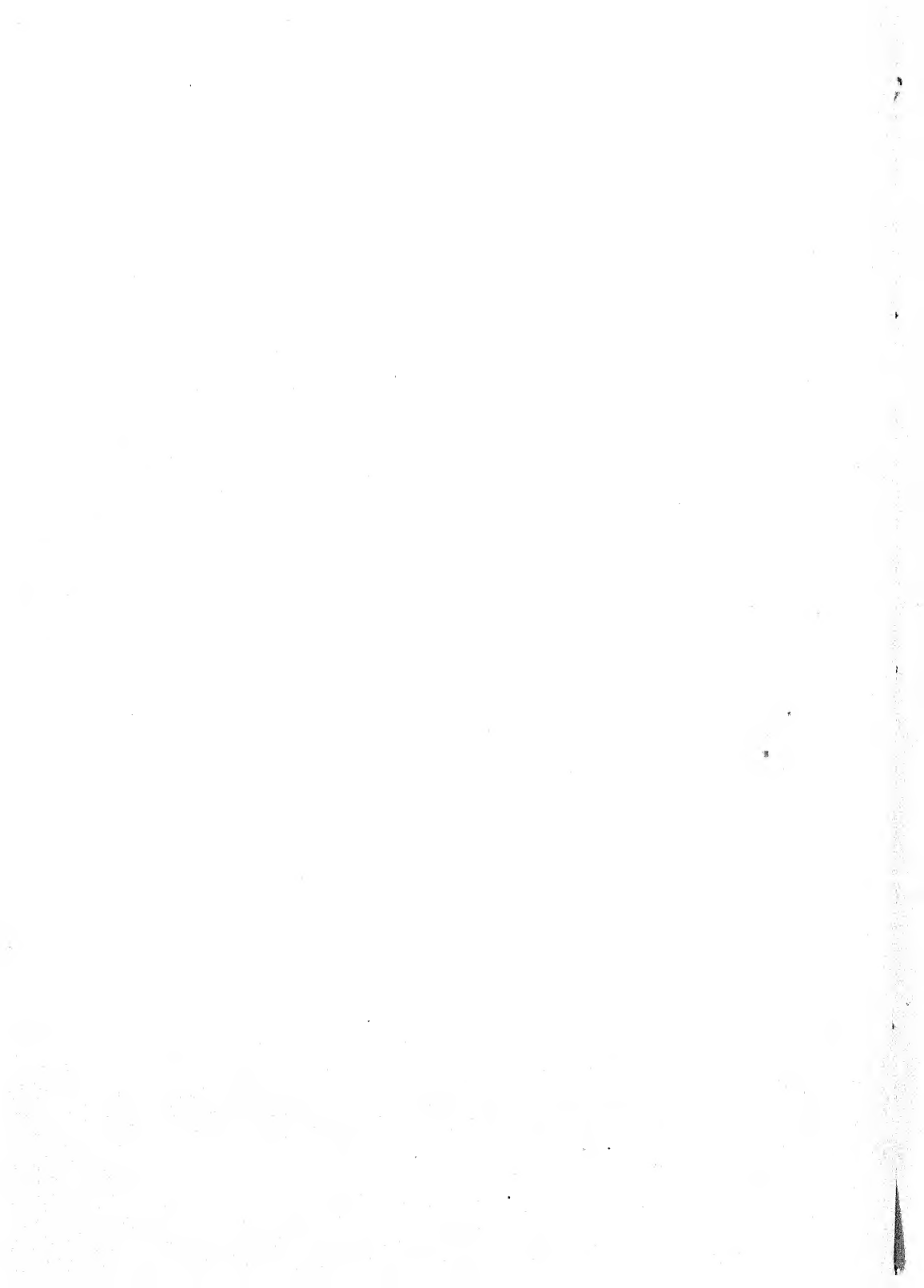
Triploidy in apples is another example of the occurrence of sterile forms in species where a substitute has been found (either in nature or in cultivation) for normal seed and fruit production. The substitution in apples is more complex than usual for, while sexual reproduction is now replaced by grafting, the necessity for the stimulus of seed growth in the formation of a fruit is largely evaded. Therefore triploids are able to fruit although incapable as a rule of yielding offspring of any value.

The offspring of triploids, whether derived from selfing or crossing with diploids, lack vigour, presumably owing to their aneuploid constitution (*cf.* Darlington and Moffett). Consequently triploid varieties are likely to be of little value in practical breeding as the necessary vigour and fertility would rarely be obtained in the resulting offspring.

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COLOUR INHERITANCE IN SHEEP.

IV. WHITE COLOUR, RECESSIVE BLACK COLOUR, RECESSIVE BROWN COLOUR, BADGER-FACE PATTERN, AND REVERSED BADGER-FACE PATTERN.

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(With Three Plates.)

I. INTRODUCTION.

A LARGE proportion of the sheep of the world would be described, loosely, as white. In certain cases, particularly in the case of breeds in which wool production is specially important, there is little pigment to be seen in skin or coat, but in other cases, although the sheep would still be described as white-fleeced, a considerable amount of pigment may be present. This may take the form of pigmented extremities, isolated single patches on the body, or scattered pigmented fibres throughout the coat. Some account of these variations was given in the third paper of the present series (1928).

In the present paper it is shown that the white-fleeced characterisation is in all probability the result of the presence in the duplex or simplex state of an inhibiting factor. In the absence of the inhibitor a range of colours and patterns appears, and it is the purpose of this paper to give an account of the inter-relationship of the white characterisation and of several of those characterisations that appear when the inhibitor is absent. It is true that other factors exist, in the presence of which sheep may be coloured or may exhibit patterns, although the inhibitor is present. Some account of one such factor, namely, dominant black, that is, a black that appears even in the presence of the inhibitor, was given in the first paper of the series (1924), and a further account in the second paper (1926). These factors are to be regarded as higher in

the series than those that will be discussed in this paper, and it is hoped at a future date to give an account of experiments involving them.

With reference to the inhibitor and to the factors lower in the series, the rather remarkable results that have emerged are of interest, because the inheritance of coat colour in the sheep appears to be rather different from the inheritance of coat colour as found in other mammals that have been studied up to the present time. But these results are of interest for another reason also. In the building up of the modern domesticated breeds, there is little doubt that much effort has been directed towards securing the presence of the inhibitor, but on the other hand there can have been little or no artificial interference with the factors inhibited by it. Consequently, under the cloak of the inhibitor have been handed on, unaffected by the hand of man, factors that may be studied in the sheep of to-day, and which, it may be hoped, will shed a good deal of light on the question of the origin of the modern types of sheep. In this connection it may be mentioned that experiments involving crosses with wild sheep are in progress at the present time.

The experimental results recorded in this paper have been obtained over a period of years covering the seasons 1923-9. During this time 386 lambs have been born during the course of experiments conducted at the farm of the University College of North Wales, Bangor; during the season 1928-9, 40 lambs at the Animal Breeding Research Department, University of Edinburgh; and a further 23 on the farms of private breeders.

II. THE NATURE AND OCCURRENCE OF THE CHARACTERISATIONS.

(a) *White.*

Variations in the white-fleeced characterisation are described in the third paper of this series (1928). Some of the white-fleeced breeds of sheep show extremely little pigment anywhere, but others may show a very considerable amount, especially on the face and legs and in the coarse fibres that may be regarded as derived from the primitive outer coat. In addition a white-fleeced sheep may possess an isolated dark patch almost anywhere on the body. The sheep which show a considerable amount of pigmentation are not truly white, but the term "white-fleeced" is sufficiently descriptive, and it appears to be clear from the results of the experiments that have been carried out that the white-fleeced condition does depend upon the same genetic constitution as regards one factor, in spite of the variations in restriction that occur.

(b) *Recessive black.*

In this paper the terms "recessive" and "dominant" are used with reference to black and brown colours in order to distinguish between the similar characterisations that result from the action of different factors. Dominant black and dominant brown are expressed even in the presence of the inhibitor, recessive black and recessive brown only in its absence. The terms dominant and recessive in this connection were applied to express the relationship of these factors to white, although no assumption was necessarily implied as regards their actual genetic relationship to white colour. The terms have been widely used, and as they should not lead to any misconception it would appear convenient to preserve them.

Recessive self-black is one of the commonest types of coat colour that crops up amongst normal white-fleeced sheep. It is subject to modification along two main lines. In the first place the tips of the wool fibres may change to a rusty brown. So far as the writers' observations go, however, the black of self-black lambs at birth is a very true black, and the change to brown only occurs subsequently. The second modification involves the admixture of varying proportions of white hairs amongst the black. In this case again it is usual for the lamb to be born black and for the greying process to take place later. The short stiff hairs of the face and legs, however, commonly preserve the original black colour, and to a large extent this is also true of the bleaching process. A type of greying is known where the lamb is born grey. This last type is uncommon and appears to be due to the action of one, or at least very few, genetic factors. It is described in particular by Adametz (1917), Dry (1924, 1927) and Wassin (1928).

The only self-blacks of independent origin used in the experiments were seven animals, viz. a Southdown ram known to be from white parents, and a Wensleydale ram and five Wensleydale ewes, secured through the kind instrumentality of Dr F. W. Dry, whose work on the occurrence of recessive blacks in the Wensleydale breed of sheep is well known.

(c) *Recessive brown.*

The occurrence of recessive brown coat colour amongst the ordinary domesticated white-fleeced breeds of sheep must be extremely rare. No case of the birth of a brown lamb in a white flock has come to the notice of the writers, nor, so far as they are aware, is such an occurrence recorded in the literature. Wassin (1928), in his paper on the numerous

colour types observed by him in Russia, does not mention the occurrence of any type of sheep which is regularly recessive brown. He does state, however, that during an inspection of the short-tailed sheep in the Government of Moscow, families were found with very dark brown pigmentation, and he also states that a dark brown pigmentation is fairly often observed in pure Merino breeding. Among some dozens of dark Merinos he only came across one which was truly black. It is very doubtful whether this brown colour referred to by Wassin is at all the same as the brown described in this paper. It may be a modification of black of a much less extreme type. Nor is it stated whether the lambs are born black and turn brown subsequently, a not uncommon phenomenon in black sheep, as has already been mentioned. In the second paper of this series (1926) a rather extreme modification of black of this type, which occurred some time after birth, was described.

Recessive brown colour, as exhibited by the sheep used in the course of the experiments described in this paper, is to be found in some rather primitive sheep in the north of Scotland, of which the Shetland and the Soay have provided material in connection with the present work. The actual sheep used were bred by Prof. Cossar Ewart, and were mainly the result of crosses between these two breeds. As regards any sheep that the writers have had the opportunity of examining, there has been no possible doubt as to whether a sheep is black or brown. It is true that black sheep often appear brownish, due to the bleaching of the tips of the wool fibres, as already described, but if the fleece is parted the black colour stands out clearly. Also the hairs of the face and legs, not so subject to modification, preserve the true black or brown colour. Dominant black and dominant brown are well known, and have been described by a number of writers. No doubt has ever been raised as to the sharp distinction between these types, and the distinction is equally sharp in the case of the similar recessive colours.

Another modification, confusing at first sight, is that black sheep may possess fibres which, especially in the case of the lamb's coat, exhibit a light brown or golden tip. Such lambs looked at from a distance appear brownish, but again if the coat is parted the essentially black colour is clearly apparent. The brownish appearance tends to disappear as the lamb grows. This modification is much commoner in sheep exhibiting badger-face and reversed badger-face patterns than it is in self-blacks. When it does occur in self-blacks, it is usually only a small proportion of fibres that are affected.

(d) Badger-face pattern.

This curious and characteristic pattern was described in the first paper of the present series (1924). It is of common occurrence in Welsh Mountain sheep, and has also been observed in Cheviots. Wriedt (1924) found it amongst the sheep of western Norway, while Heller (1915) describes the occurrence of a badger-face lamb in a Rambouillet flock, and states that it is to be found amongst the woolless sheep of Barbados, and in crosses of this breed with the Southdown. The pigmy sheep from the Cameroons, exhibited in the British Museum (Natural History), shows the badger-face pattern, and this specimen is described by Lydekker (1912), who does not mention the occurrence of this colour type in any of the other sheep described in his book. Wassin (1928) states that during the course of his observations on sheep in Russia this pattern was found three times amongst the short-tailed sheep of the Government of Moscow, and four times during the sorting of Karakul lamb pelts in Uzbekstan.

Badger-face pattern is subject to much modification, and during the course of the present experiments sheep have been examined which form a complete unbroken series, ranging from those which show just a very little pigment on the face and legs, arranged in the characteristic fashion, to those which are practically black all over. In extreme cases it might be very difficult to distinguish between a very dark badger-face and a self-black. In those cases during the course of the experiments where from the appearance of the animal it was difficult to make this distinction, that fact is noted in the table.

Characteristic modifications of the pattern appear to be typical of certain flocks and strains of Welsh sheep, so much so that in a mixed lot of badger-faces it is sometimes possible with experience to tell at a glance the particular flock from which a sheep was obtained. A large proportion of the sheep used in the experiments were drawn from the mountain flock of the University College of North Wales where the characteristic badger-face pattern is on the dark side; hence the number of dark badger-faces that cropped up was fairly considerable.

In the previous paper (1924) photographs were included of the typical pattern and also of the very light modification. Plate VI in the present paper shows dark badger-face sheep exhibiting varying amounts of black. The sheep illustrated in Figs. 5 and 6 on this plate was one of the darkest obtained, and it is to be noted that the little spots of white underneath the eye appear to be characteristic of the very dark badger-face.

Since practically all the sheep involved in the experiments have been genetically blacks and not browns, there has been no opportunity as yet for the production of a brown and white badger-face, but there seems to be no reason to suppose that it could not be obtained just as easily as a brown and white reversed badger-face.

(e) *Reversed badger-face pattern.*

A preliminary description of this pattern was given by Roberts and Jenkin (1926). It is very similar to the pattern shown by some wild sheep such as the Mouflon. The dark body, light ventral surface, and the characteristic markings of the head and elsewhere are also very similar to a general type of pattern of quite common occurrence amongst many Ungulates.

In the case of the original stock of sheep of this type at Bangor, all were black and white and were of the Welsh Mountain breed. They were obtained in mid-Wales, and it is interesting to note that there was some history of crossing with sheep from Scotland a considerable time ago. One sheep, however, was of unknown origin, being found in a commercial flock. During the course of travels in Wales the writers have occasionally observed reversed badger-face sheep, but the proportion occurring is exceedingly small compared with the proportion of badger-face sheep found in the Welsh Mountain breed. Reversed badger-face pattern appears to be characteristic of the Soay sheep, and was exhibited by a number of the sheep bred by Prof. Cossar Ewart already referred to. Wassin (1928) in the course of his extensive study, does not mention the occurrence of the reversed badger-face type.

The same considerations relative to the distinction between black and white and brown and white reversed badger-face apply as in the case of the distinction between self-black and self-brown.

The similarity between this pattern and badger-face pattern is very striking. In general it may be said that those areas which are coloured in the badger-face are white in the reversed badger-face and *vice versa*. Typical representatives of the two sorts of pattern show this correspondence down to very small details. Figs. 4 and 5, Plate VII, show a typical reversed badger-face lamb, and to contrast with this, Fig. 2, showing a typical badger-face lamb, is also given. Figs. 6, 7 and 8 show a typical reversed badger-face ewe. Fig. 3 shows the head of the reversed badger-face ram used in the course of the experiments, and to contrast with this, Fig. 1 is given—a photograph of the head of a typical badger-face ram also used in the course of the experiments.

It appears to be characteristic of reversed badger-face pattern, as contrasted with badger-face pattern, that it is less liable to extreme modification. Whether this is merely true of the sheep the writers have had the opportunity of observing, or whether it is more universally true, is doubtful, but in all probability there is some distinction in this respect. Fig. 2, Plate VIII, shows one common type of modification of the reversed badger-face pattern, but it will be seen that it is not a particularly extreme type of modification. Fig. 5 on this plate is a photograph of the only lamb that occurred during the course of the experiments which showed really extreme modification. The breeding of this lamb leaves little room for doubt that it is to be regarded as a reversed badger-face, but it would be rather difficult to distinguish it from a very dark badger-face.

One modification has already been mentioned in connection with recessive black, namely, the occurrence of fleece fibres with a light brown or golden tip. Wassin describes an agouti type of coloration, and was kind enough to send the writers a sample. It is, however, of a different nature. Wassin's agouti fibres show a light band, together with a dark tip, whereas this was never observed in the sheep described in this paper. In all cases the banding of the hair involved a light tip only. It appears to be characteristic of badger-face and reversed badger-face patterns that the occurrence of parti-coloured hairs is far more common than it is in the case of self-black, and that where they do occur they are far more likely to occur in large numbers. Figs. 1 and 3, Plate VIII, show a reversed badger-face lamb with the most extreme development of parti-coloured hairs that the writers have observed. A casual glance would lead to the assumption that this lamb was brown, but when the fleece was parted the essentially black character was revealed. This lamb may be contrasted with the lamb shown in Fig. 4 of the same plate, which is a brown and white reversed badger-face. In this case when the fleece was parted the brown colour extended to the base. Wassin has also observed that his type of agouti pattern appears to be associated with badger-face pattern. All the badger-face sheep observed by him, viz. seven, were also agouti. He suggests tentatively that the badger-face pattern and agouti pattern may be linked. The writers are not quite clear whether he means to imply linkage in the genetic sense or merely association. A far more probable explanation than true genetic linkage would appear to be that parti-coloured hairs develop more readily in animals showing badger-face or reversed badger-face patterns than they do in self-blacks, i.e. that there is a different threshold for pigment formation.

III. EXPERIMENTAL RESULTS.

The table on the opposite page gives in summarised form the results of the breeding experiments carried out during the years 1923-9¹.

It is to be noted in the first place that there is no evidence of sex linkage. Secondly, in all cases homozygous dominants cannot be distinguished, as far as ordinary observation goes, from heterozygous dominants, *i.e.* dominance is complete. It will be seen from the table that white crossed to any other form gives white, badger-face crossed to reversed badger-face or self-colour gives badger-face, reversed badger-face crossed to self-colour gives reversed badger-face, and black crossed to brown gives black.

In the case of two badger-face sheep heterozygous for that factor and either homozygous or heterozygous for the reversed badger-face factor, there was a slight variation in that the chin was whiter than in ordinary badger-face sheep. These animals gave badger-face lambs when mated to sheep lower in the series. It is possible that this slight modification might be due to the presence of the reversed badger-face factor in a badger-face sheep, but all the other numerous animals of similar genetic constitution appeared indistinguishable from ordinary badger-faces.

At an earlier stage during the course of these experiments it was thought that multiple allelomorphism might be involved, but it is now clear that this cannot be the case. White, black and brown cannot form a system of multiple allelomorphs because, as the table shows, the white F_1 from a white-brown cross can yield black in F_2 . On the hypothesis of multiple allelomorphism the white F_1 's could not carry a black factor. This conclusion is confirmed by the results of experiments carried out by Cossar Ewart (1919) in which the brown Soay was crossed to the Southdown. The F_1 was white. In F_2 , and also in F_3 using the white F_2 animals, blacks appeared. It is also clear that the two patterns cannot be involved in any multiple allelomorph system. It will be seen from the table that white \times reversed badger-face gave white. A back-cross to reversed badger-face gave, in addition to white and reversed badger-face, three badger-faces. In the same way white \times black gave white, and the F_1 back-crossed to black gave, in addition to whites and blacks, one badger-face.

The following scheme fits the results:

¹ Full tables of the matings have been prepared and are being deposited for reference at the British Museum (Natural History).

TABLE.

No. of full table		White	Badger- face (black)	Reversed badger-face		
				Black	Brown	Brown
II	White × badger-face (black)	31	3	.	.	.
III	White-badger-face (black) F_2	8	1	.	.	.
IV	White-badger-face (black) F_1 × badger-face (black)	4	10	.	.	.
V	White-badger-face (black) F_1 × recessive black		3	.	.	.
VI	White × reversed badger-face (black)	32
VII	White-reversed badger-face (black) F_1 × reversed badger-face (black)	3	3	.	.	.
VIII	White-reversed badger-face (black) F_1 × recessive black	7	1	2	.	.
IX	White ♂ × white ♀, all of stock giving many reversed badger-faces (black)	10	.	5	.	.
X	White × reversed badger-face (brown)	12	1	.	.	.
XI	White-reversed badger-face (brown) F_2	4	.	1	.	.
XII	White × recessive black	67	7	.	.	.
XIII	White-recessive black F_1 × recessive black	9	1	.	.	5
XIV	White × recessive brown	9
XV	Badger-face (black) × badger-face (black)	.	36	.	.	.
XVI	Badger-face (black) × reversed badger-face (black)	.	23	1	.	.
XVII	Badger-face (black) × reversed badger-face (black) F_1 × reversed badger-face (black)	.	14	13	.	1
XVIII	Badger-face (black) × reversed badger-face (black) F_1 × recessive black	.	10	2	.	1
XIX	Badger-face (black) × reversed badger-face (black) F_1 × recessive brown	.	.	1	.	.
XX	Badger-face (black) × recessive black	1	21	.	.	.
XXI	Badger-face (black) × recessive black F_1 × recessive black	.	9*	.	.	11
XXII	Badger-face (black) × recessive brown	.	5*	.	.	.
XXIII	Reversed badger-face (black) × reversed badger-face (black)	.	1†	5	.	.
XXIV	Reversed badger-face (black) × recessive black	.	.	3	.	.
XXV	Reversed badger-face (black) × reversed badger-face (brown)	.	.	11	.	.
XXVI	Reversed badger-face (black) × recessive brown	.	.	4	.	.
XXVII	Reversed badger-face (brown) × recessive brown	.	.	1	.	4
XXVIII	Recessive black × recessive black
XXIX	Recessive black × recessive brown	5
XXX	Recessive brown × recessive brown	17

* In both these cases two lambs were very dark and may have been blacks.

† A badger-face ram broke into the flock at a time corresponding to the birth of this lamb.

1. White colour depends upon the presence of an inhibitor, which prevents the appearance of self-colour or of the patterns.

2. If the inhibitor is absent the coat may be either black or brown. Whether black is allelomorphic to brown or not must be left an open question. This point will be discussed further in this section.

3. In the absence of the inhibitor the factor for badger-face pattern either in the duplex or simplex state gives badger-face. Brown and white badger-faces have not yet been obtained, but there is no reason to suppose that they cannot be produced. If the reversed badger-face factor is also present, either in the duplex or simplex state, it produces no effect; badger-face sheep which possess in addition the reversed badger-face factor appear to be indistinguishable from badger-face sheep that do not possess it.

4. In the absence of the inhibitor and also in the absence of the badger-face factor, the reversed badger-face factor in either the duplex or simplex state turns self-colour into reversed badger-face.

The true genetical significance of a characterisation at the bottom of a series must of course always be uncertain, and there are three possibilities which would explain the observed results in addition to the above simple scheme:

1. White and black might be allelomorphic, in which case the appearance of brown colour would be due to the action in the homozygous condition of a recessive factor which turned black into brown.

2. White and brown might be allelomorphic, in which case black colour is the result of the action of a dominant factor turning brown into black.

3. Black and brown might be allelomorphic, in which case white is the result of the presence in the duplex or simplex state of a dominant inhibitor.

There is nothing in the actual figures obtained that would enable a decision to be made on the relative merits of these possibilities. It can merely be stated that on grounds of analogy it is unlikely that white is allelomorphic either to black or brown. An ordinary white sheep may exhibit extremities of a tan colour and at the same time a single patch which is as black as the black of a self-coloured animal. The probability is that the ordinary white sheep is a black sheep which also possesses the inhibiting factor restricting the pigment to a greater or lesser extent. It is also probable that a white sheep might be fundamentally brown. There is no evidence whatsoever to show whether black and brown are allelomorphic or not, and until a new characterisation is produced it is

probable that this question will remain unsolved. The analogy with dominant black and dominant brown may, however, be usefully considered. In that case it has been shown by Duck (1921, 1922), Adametz (1917) and Wassin (1928) that dominant black and dominant brown are not allelomorphic, and that dominant brown depends upon the presence of a factor in the duplex or simplex state, which can only express itself if the factor for dominant black is absent.

One further possibility must be considered, namely, that brown colour is due to the action in the duplex state of a recessive factor which turns black into brown. This is the hypothesis advanced by Wassin to account for the occurrence of the browns noted by him that have already been discussed, and which, it was pointed out, are probably not the same brown as the brown sheep described in this paper. Again, it will in all probability be impossible to determine whether this hypothesis is or is not correct until a new characterisation lower in the series is discovered. As far as primitive sheep can be classified as browns or blacks, brown appears to be the usual colour. This being the case, it is perhaps rather unlikely that brown should depend upon a modification of the black characterisation. It is more probable that black is a special additional feature found in domestic sheep, and that brown colour depends upon the presence of a definite factor.

One important point must be made. Whether the simple scheme suggested is accepted, or whether one of the alternative modifications is preferred, the contention is still valid that under the cloak of the white-fleeced condition of the ordinary domestic sheep exists a range of colours and patterns that can be studied in suitable crosses.

It is now possible to analyse further the results of the table.

Classifying the sheep as white and non-white, the following figures are obtained:

(1)	White	Non-white
White \times non-white	151	12
White-non-white F_2	12	2
White-non-white back-cross	23	28
Non-white \times non-white	1	205

It is clear that the assumption of a single factor difference between white sheep and the non-white sheep dealt with in this paper is adequate. The one white in the non-white \times non-white matings requires a word of explanation. This was a lamb born in a badger-face (black) \times recessive black mating. It was twin to a badger-face lamb. Unfortunately this white lamb was not kept for further examination at a more adult stage.

It is probably unlikely that any mistake could have occurred¹. Of course the assumption of a different type of white would explain the colour of this lamb, and this is referred to later, but the writers would prefer to leave the question open.

(2)	White	Badger-face	Non-badger-face
Badger-face × badger-face	.	36	.
Badger-face × non-badger-face	1*	49	2
Badger-face-non-badger-face back-cross	.	33†	28
Non-badger-face × non-badger-face	.	1‡	56

* The same anomalous white as that recorded in the non-white × non-white cross above.

† Four were very dark and may have been blacks.

‡ Explained by the breaking in of a badger-face ram at a time corresponding to the birth of this lamb.

One point of great interest emerges from these figures. In view of the extreme range of modification exhibited by badger-face pattern, it might be thought that the various grades of the pattern depended upon the action either singly or in combination of more than one factor modifying self-colour. The figures for the back-cross, however, afford no support to such a hypothesis, but point clearly to the fact that badger-face pattern depends essentially upon the presence of a single factor, and that the modifications observed are to be ascribed to the action of modifiers that only exert their effect in the presence of this main factor.

One experiment involving the various grades of badger-face pattern was carried out. A ram exhibiting the typical pattern was mated to a number of ewes showing various amounts of pigment. There was a well-marked tendency for the lambs to approach closer to the typical pattern than their mothers, or rather to tend to approach a pattern a little darker than the typical. This was not surprising, because the ram, though practically typical himself, was from an ancestry with a strong tendency to give rather dark badger-faces.

(3)	Badger-face	Reversed badger-face	Non-reversed badger-face
Reversed badger-face × reversed badger-face	1*	16	.
Reversed badger-face × non-reversed badger-face	.	8	4
Non-reversed badger-face × non-reversed badger-face	.	.	28

* The same lamb as that recorded in the non-badger-face × non-badger-face mating of the previous text table. A mistake.

The occurrence of a rather high proportion of non-reversed badger-faces in the mating reversed badger-face × non-reversed badger-face is

¹ It is known that a sheep just before parturition may steal the lamb of another sheep. As regards the great bulk of the results recorded the possibility is extremely remote, owing to the careful attention of the shepherd; but this lamb was born in a lot that was kept for lambing at an auxiliary farm where the same detailed care was not available.

due to the inclusion in the experiments of Prof. Cossar Ewart's sheep. Owing to the Soay influence in this flock, a proportion were reversed badger-faces, but attempts had been made to reduce this number. Consequently it is almost certain that all the reversed badger-faces in this flock were heterozygous for that factor.

(4)	White	Black	Brown
Black \times black	1*	157	.
Black \times brown	.	26	.
Brown \times brown	.	.	22

* This is the same lamb as that recorded in the non-white \times non-white mating above (p. 175).

The writers have had the opportunity of obtaining data from the Shetland flock of Mrs Campbell, Dolphinton House, Lanarkshire. This flock consists mainly of self-browns, but in addition self-blacks, brown reversed badger-faces and black reversed badger-faces occur. All the blacks are descended in an unbroken line of blacks from three black ewes in the foundation stock. In no case has a black lamb been produced except from a black parent, and the result of a brown \times brown mating has invariably been the production of browns.

Elwes (1913) states that in his flock of 25 brown Shetland sheep he did not have a single black, spotted or white lamb. Ewart (1919) states that the Siberian Mouflon also has a brown fleece, and when crossed with Cheviots gives white offspring. Brown sheep were obtained in the F_2 generation, which when back-crossed to the Siberian Mouflon ram gave pure-breeding brown sheep.

If black and brown should prove not to be allelomorphic, an interesting possibility is raised. If a sheep possesses neither the brown factor nor the black factor, what will be its characterisation? Presumably it will be white, unless a factor or factors for colour in general are postulated. On grounds of analogy with other animals there seems to be no reason why a recessive white should not exist. Wasson (1928) gives an anomalous result which, he states, might be explained by postulating a recessive white, but makes no assumption that such a colour type does exist. Mr F. Darling, of the Animal Breeding Research Department, Edinburgh, has made some observations on Shetland sheep in Shetland, and the writers are indebted to him for the information that there appears to be the possibility of white sheep resulting from the interbreeding of coloured animals, the colours presumably being all of the recessive type. In the absence of any definite experimental evidence the question must be left open, but an attempt will be made by raising

an F_2 from black and brown sheep, to explore it further. Should this new sort of white be produced it would possibly be a matter of some economic significance, as it is known that type of pigmentation and type of structure of wool are often associated, so that a recessive white sheep might possess a fleece of different structural characteristics from the common type.

IV. ACKNOWLEDGMENTS.

During the season 1928-9 the very interesting sheep bred by Prof. Cossar Ewart used were at the Animal Breeding Research Department to provide data in connection with these experiments, and 40 lambs were born at Edinburgh in 1929. In addition a brown ram from this flock was used at Bangor. The writers are deeply grateful to Prof. F. A. E. Crew and to Mr W. C. Miller for the invaluable facilities provided.

Mr T. J. Jenkin, of the Welsh Plant Breeding Station, Aberystwyth, co-operated with one of the writers in preparing the preliminary description of reversed badger-face pattern. Mr Jenkin was instrumental in securing all the original stock of reversed badger-faces except one. The writers wish to acknowledge most gratefully the help he has given, and also that of Mr M. B. Jones, Cyneiniog, Cardiganshire, who provided most useful information regarding the occurrence and breeding of this type.

Mr T. L. Bywater, now of the Department of Agriculture, University of Leeds, rendered most valuable assistance during the spring of 1927 and again in 1929 in recording the birth of the experimental lambs.

The kind help of Dr F. W. Dry, who secured black Wensleydale sheep for the experiments, has already been mentioned in the text.

V. SUMMARY.

1. The white-fleeced condition in sheep depends upon the presence of a dominant inhibitor.

2. In the absence of the inhibitor the coat may be either black or brown. (These two colours are called recessive colours to distinguish them from the similar characterisations that are due to the action of other factors that produce their effect even in the presence of the inhibitor.) Black is either allelomorphic to brown and dominant to it, or else the brown factor is not expressed in the presence of black. Alternatively brown might be regarded as a modification of black.

3. Badger-face pattern depends upon the presence of a dominant

factor acting on recessive self-colour. It produces no effect if the inhibitor is present.

4. Reversed badger-face pattern depends upon the presence of a dominant factor acting on recessive self-colour. Again, it produces no effect if the inhibitor is present. If both badger-face and reversed badger-face factors are present, the badger-face factor alone is expressed, the reversed badger-face factor producing no effect.

5. As the bottom member of the series, the genetics of brown colour cannot be exactly known at present.

6. Several modifications are discussed, as well as the remarkable reversal of colour in the case of the two patterns.

7. It is pointed out that under the cloak of the inhibitor modern white-fleeced sheep possess colour factors that can have been little affected by artificial selection.

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EXPLANATION OF PLATES VI—VIII.

PLATE VI.

Dark badger-faces.

PLATE VII.

Fig. 1. Badger-face ♂ 8 (1924).

Fig. 2. Typical badger-face lamb.

Fig. 3. Reversed badger-face ♂ P 6.

Figs. 4, 5. Typical reversed badger-face lamb.

Figs. 6-8. Typical reversed badger-face ♀.

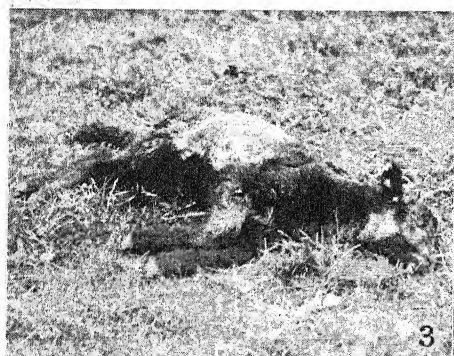
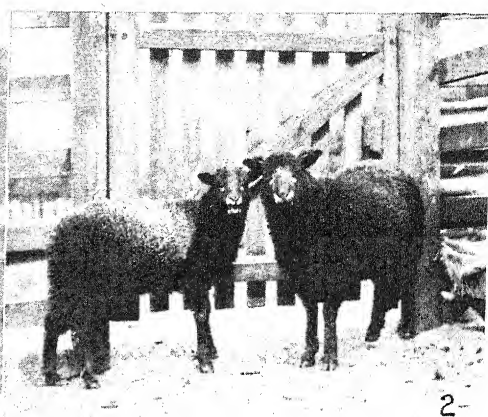
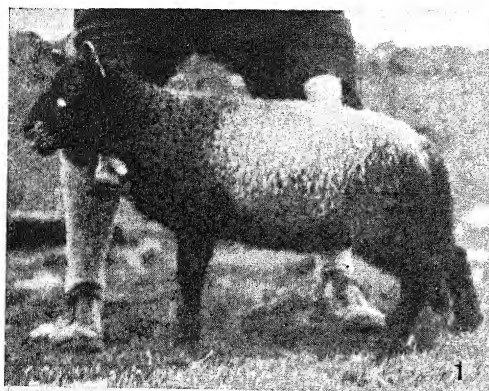
PLATE VIII.

Figs. 1, 3. Black reversed badger-face lamb showing development of parti-coloured coat fibres.

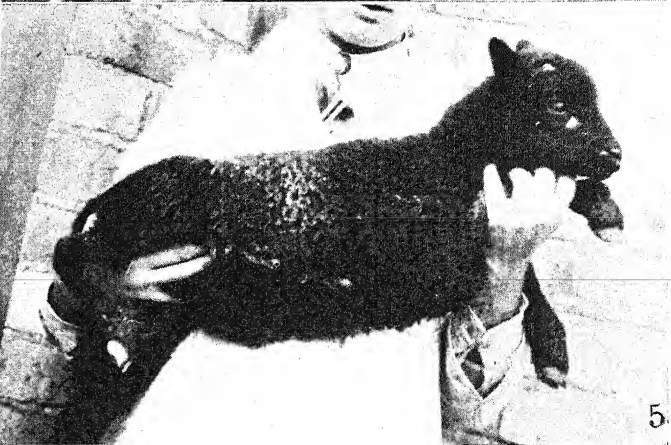
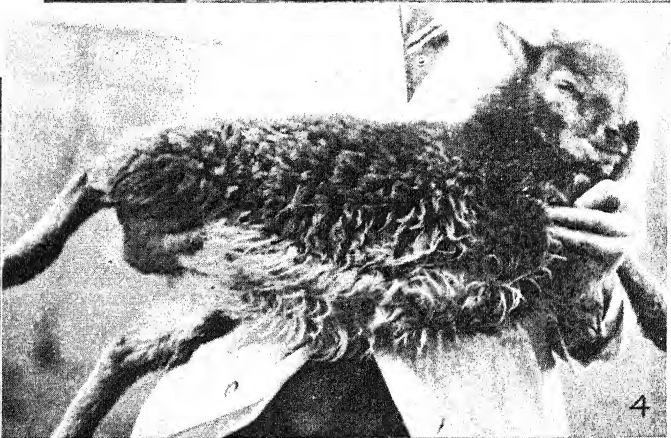
Fig. 2. A common type of modification of reversed badger-face pattern.

Fig. 4. A brown and white reversed badger-face lamb.

Fig. 5. Very dark reversed badger-face lamb. The only case of extreme modification.







COLOUR INHERITANCE IN SHEEP.

V. DOMINANT BLACK.

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I. THE BLACK OF THE BLACK WELSH MOUNTAIN BREED.

In the first paper of this series (1924) a description was given of this breed and of an F_1 resulting from a cross with ordinary white sheep. A black ram of this breed crossed to white ewes gave:

23 blacks,

and black ewes crossed to a white ram gave:

5 blacks and 2 whites.

An F_2 generation was raised, the result being:

18 blacks and 2 whites¹.

Three of the F_1 females used were actually from a black badger-face² mating. In view of the results given in a later section of this paper it is justifiable to include them, the crosses actually being black \times non-black.

In view of the wide divergence between the figures for the F_2 generation and the expected 3 : 1 ratio, it was decided to back-cross the F_1 females to white. The result was:

28 blacks and 22 whites³.

As already explained, it is possible to treat a black badger-face cross as black \times non-black, so that the result of a black badger-face back-cross given later can be added. The total result now becomes:

39 blacks and 29 non-blacks.

¹ Full tables of the various matings have been prepared and are being deposited for reference at the British Museum (Natural History).

² For description of badger-face pattern see papers I and IV of this series.

³ These sheep were taken over by a private breeder. Totals only were recorded each season. Additional results 1930—4 blacks and 8 whites.

The back-cross ratio is fairly normal, but the slightly larger class is still the black one. The possibility that there is more than one factor that results in dominant black is considered in the next section.

II. THE OCCURRENCE AND INTER-RELATIONSHIP OF DOMINANT BLACKS.

Dominant black, *i.e.* a black dominant to white, is one of the best known colour types in sheep. The black of the Karakul breed has been described by Adametz (1917), Duck (1921, 1922), Wassin (1928) and others, and shown to be dominant to white. It is also known in other breeds of mid-Asia. One of the writers in the second paper of this series (1926) described experiments demonstrating the existence of a dominant black in the Piebald breed of sheep, a number of flocks of which breed are in existence in Great Britain. In this case the black is turned into piebald by the presence in the duplex state of a recessive pattern factor. A rather similar case is that of the Somali and the Persian Fat-tail. In these breeds, as shown by the results of Henseler (1913) and Davy (1927), the dominant black is turned into a pattern in which the head and neck only are pigmented. This is due to the presence of a factor in the duplex state; in the simplex state the factor turns colour into a variety of piebald. Wassin states that this pattern also occurs in Mongolian sheep. He explains the results of Henseler and Davy on the above basis, and also gives the results of his own experiments confirming that conclusion.

The origin of the dominant black of the Black Welsh Mountain breed is a question of considerable interest. The breed is a small one and the history usually obtained is that it arose by the selection of black individuals from ordinary white Welsh Mountain flocks. It is perhaps not inconceivable that the dominant black is the result of the introduction at some time of black Asiatic sheep, or, of course, crosses with the Piebald breed could have introduced it. An Asiatic origin appears to be very unlikely because in those breeds the sheep, in addition to the black factor, often carry a factor for dominant brown (which factor only expresses itself in the absence of dominant black). Dominant browns do not occur in the Black Welsh breed. Heterozygosity in this breed is a marked feature, but the recessives appearing are white. The possibility of the black being introduced owing to crosses with the Piebald cannot be so easily dismissed. This must be regarded as a perfectly reasonable theory. Perhaps the most probable explanation is, however, that dominant black does occur amongst English breeds, though as the various breeds have been improved it would tend to disappear. In

ordinary commercial flocks in which a few blacks are to be found there is often a history of these sheep having had black mothers. There seems to be no reason why in certain cases the dominant black cannot still occur, being handed down in an unbroken succession of blacks. Although a black ram would not be used in many commercial flocks, no attempt would be made to get rid of black ewes.

In order to test the relationship of the black of the Welsh Mountain breed to that of the Piebald breed, crosses were made. The F_1 lambs were all blacks. An F_1 ram was mated to white ewes, the result being:

23 blacks, 2 piebalds and 1 white.

As the piebalds are genetically blacks, this result is really:

25 blacks and 1 white.

No doubt is possible in the case of the one white. This was co-twin to a black, and in addition the characteristics of the Piebald breed are so distinctive that the lambs of the F_1 ram could readily be distinguished from any others in the whole countryside.

It is difficult to explain these figures. It was thought that one of three results would be obtained:

(1) That the factors would be identical and the result all blacks.

(2) That the factors would be different, giving a result of 3 blacks to 1 white.

(3) That one parent of the F_1 ram might have been heterozygous, and not have handed on the black factor, giving a result of 1 black to 1 white.

Taking these figures, however, in conjunction with the abnormal F_2 ratio given in the preceding section, the possibility is undoubtedly raised that there may be more than one dominant black factor. If there were two black factors in the Black Welsh and a third in the Piebald, the F_1 ram might be a triple heterozygote, the expectation in a cross with white being 7 blacks : 1 white. However, this can only be a speculation and the question must be left an open one. Nevertheless, taking the result of this section and that of the preceding one together, it does seem possible that more than one dominant black factor is involved.

These experiments, together with those previously recorded, indicate that the piebald factor is not of very rare occurrence amongst ordinary white sheep. The question arises as to why piebald lambs are not sometimes born, an occurrence that must at the best be extremely rare. It is, of course, possible that the piebald factor does not express itself in the case of recessive colour, but only in the case of dominant colour.

It is probably, however, not necessary to make this assumption. Given random mating, if

m = percentage heterozygotes in the parental generation,

and x = percentage recessives appearing in the filial generation,

$$x = \frac{m^2}{400}.$$

If 10 per cent. of a sheep population were heterozygous for the inhibitor, 0.25 per cent., or 1 in 400 recessive blacks would appear amongst the lambs.

If, however, the characterisation depended upon the simultaneous presence of two recessive factors, the proportion occurring is greatly reduced. The formula now is

$$x = \frac{m^2 n^2}{16 \times 10^4},$$

where x = percentage of double recessives in the filial generation,

m = percentage heterozygotes as regards one factor in the parental generation,

and n = percentage heterozygotes as regards the other factor in the parental generation.

Assuming a heterozygosity as high as 10 per cent. for the piebald factor as well as for the white inhibitor, the percentage piebalds appearing would only be 0.0006 per cent., or 1 in 160,000. If a 5 per cent. heterozygosity is assumed for both factors, the proportion of double recessives appearing would be only 1 in 2,560,000.

III. THE RELATIONSHIP OF DOMINANT BLACK TO RECESSIVE BLACK AND TO BADGER-FACE AND REVERSED BADGER-FACE PATTERNS.

In the first paper of this series the F_1 of a cross between dominant black (Welsh Mountain) and badger-face was described. A black ram crossed to badger-face ewes gave:

12 blacks and 1 white.

Black ewes crossed to a badger-face ram gave:

3 blacks and 1 badger-face.

The occurrence of the single white is discussed in a subsequent section.

A black F_1 ram from the above mating was back-crossed to badger-face ewes, the result being:

11 blacks, 3 whites and 4 badger-faces.

This is a close approximation to the expected 2 : 1 : 1 ratio on the basis of a two-factor difference. The black sheep possess the white inhibiting factor as well as the black factor.

Reversed badger-face sheep (see fourth paper of the series) were also crossed to dominant black, the ram used in this case being a Piebald. The result was:

5 blacks.

The same Piebald ram crossed to badger-faces gave:

3 blacks.

These results are of interest in view of the information they provide as to the relationship of dominant to recessive black. As regards ordinary visual inspection no distinction in appearance can be made out, and Wassin (1928) states that the rate of bleaching with hydrogen peroxide is the same for black wool taken from sheep of both kinds. While, however, the factors for badger-face and reversed badger-face patterns turn recessive self-colour into the corresponding pattern, they produce no effect at all on dominant black. If, following Onslow (1915), white colour is regarded as being due to the presence of an inhibiting enzyme, dominant black colour cannot simply depend upon an anti-inhibitor, because if this were the case the result of a dominant black-badger-face cross would be badger-face. It is more probable that the effect of the dominant black factor is to intensify the processes resulting in the formation of black pigment, and this to such a degree that neither the inhibitor nor the two pattern factors can produce any effect.

IV. MODIFICATIONS OF BLACK COLOUR.

A brief note on the modifications of black colour, apart from the effect of main pattern factors such as badger-face, was given in the fourth paper of this series.

One of the largest modifications is the case where lambs are born grey owing to an admixture of white hairs with the black. Dry (1924, 1927) describes such a type, which he calls silver-grey, and shows it to be due to the action of a single dominant factor. Wassin (1928) describes a similar modification also due to the effect of a single dominant. Dry's sheep were recessive blacks, while Wassin only discovered this factor amongst the dominant blacks of southern and south-eastern Russia, nevertheless it is possible that both observers are dealing with the same factor. This modification was not observed during the course of the experiments.

On the other hand a type of greying was observed amongst the heterozygotes, this process not occurring to any extent until the animal is mature, and increasing gradually with age. Black Welsh sheep are naturally selected for a deep uniform black, so it is not to be expected that such a modification will be of wide occurrence amongst them. It was in all probability introduced by the white parents. Wassin describes the probable existence of two factors causing a greying later in life, one of them, however, causing a profound change in about six months when in the homozygous condition. No *ad hoc* experiments were carried out by the writers on this problem, so the existence of the modification in the experimental sheep is simply recorded.

It is often observed that lambs that are born black turn brownish later, the tips of the wool fibres especially appearing to bleach. Many degrees of change may be observed, but one special case occurred during the experiments that appeared to be of a more definite character. It was stated in the second paper of the series (1926) that the pigmented areas of the Piebald sheep of the flock from which were drawn the experimental animals rapidly lost the original very dark colour and became a definite dark fawn. It was noted that in the self-black F_1 's resulting from crosses with this breed the process did not occur. Ten lambs were born in connection with a back-cross of the F_1 to Piebald. At the time the above paper was written it was decided that the lambs should be observed for a further period, and the result as regards the bleaching of the fleeces is now given. This was:

4 blacks (like ordinary blacks);

5 bleached (like Piebald breed);

1 doubtful—this animal had brownish shoulders and dark hind-quarters.

There is thus the suggestion that in this case a single recessive factor may be responsible for the marked modification. It is possible that this is the same factor as that described by Wassin—also recessive—that turns black into brown.

V. AN ANOMALOUS RESULT.

In the first paper of the series (1924) it was recorded that the dominant black ram used, when mated to white ewes, sired 23 blacks, and when mated to badger-face ewes, 12 blacks and 1 white. He thus gave 35 blacks and 1 white. It was decided to test further the single anomalous white. Mated to white ewes this anomalous ram gave:

7 whites.

Mated to badger-faces he gave:

10 whites.

Apparently, therefore, he was not even heterozygous for the white inhibitor, although his mother was a badger-face and could not have possessed it.

This lamb was co-twin to a black. The possibility of a double fertilisation by two rams may be ruled out. Not only is it practically impossible that a strange ram could have broken into the field without the knowledge of the shepherd, but the black ram was an animal of such ferocity that he would undoubtedly have killed such an intruder. One possible explanation is that an event occurred which is known to happen in the sheep, although owing to the care of the shepherd the chance is extremely remote in this particular flock, viz. that the ewe just before parturition took away the lamb of another sheep.

VI. A BRIEF SUMMARY OF COLOUR INHERITANCE IN THE SHEEP.

A brief summary of what is known at present of the inheritance of coat colour in the sheep may not be out of place. Sheep may be of five fundamental colour types:

- | | |
|---------------------|----------------------|
| (1) Dominant black. | (4) Recessive black. |
| (2) Dominant brown. | (5) Recessive brown. |
| (3) White. | |

Each factor in the duplex or simplex state prevents the appearance of any characterisations lower in the series. The exact relation of the two bottom members of the series must remain doubtful for the present; they may be allelomorphic.

The main known modifications of these basic colours are as follows:

1. A recessive piebald factor which in the duplex state turns dominant black into piebald. Its effect on the other colours is not known. It has probably no effect on white.

2. A factor which in the duplex state turns dominant black or dominant brown into black-headed pattern; in the simplex state into a form of piebald. Its effect on the recessive colours is not known.

3. The badger-face factor, which in the duplex or simplex state turns recessive black and probably recessive brown into badger-face pattern. It has no effect on white or the dominant colours.

4. The reversed badger-face factor, which in the duplex or simplex state turns recessive black or recessive brown into reversed badger-face.

It can only be expressed in the absence of the badger-face factor. It has no effect on the dominant colours or on white.

In addition to the above, other factors are known which produce less profound effects, while in yet other cases the genetics of the modifications are not fully known. Wassin may be consulted for a fuller account.

The most important of these modifications about whose genetics something is known are as follows:

1. A white patch on the top of the head and a white tip to the tail is a modification that appears to crop up amongst all coloured sheep. Adametz (1917) considered it to be based on the presence of a recessive factor, but Wassin gives data which show that it is a dominant. Many white sheep carry this factor, which in them cannot of course be expressed. Many of the coloured sheep in the writers' experimental flocks exhibited this pattern.

2. Wassin describes a pattern prevalent amongst the black Romanov sheep and also observed by him in other recessive blacks. This pattern involves varying amounts of white on the face and legs. It is possible that the sheep exhibiting a little white only may be identical with those showing the white pattern described above, but by no means certain in view of the relationship to dominant black. The various grades of this pattern appear to depend upon a series of multiple factors. Wassin assumes five in order to explain his results. It is very probable that these factors do not affect dominant black.

3. A dominant factor which in a coloured sheep produces a white collar. This characterisation and its inheritance are described by Wassin. It may be expressed in dominant or recessive blacks.

4. Data were given in the third paper of the present series regarding the probable existence of two pairs of factors which affect the amount and distribution of pigment on the face and legs of white-fleeced sheep. These factors do not affect coloured sheep.

5. A dominant factor which turns black into grey. Lambs are born grey. This modification is fully described by Dry, and may be identical with the similar factor also fully described by Adametz and by Wassin.

6. Greying later in life is more complicated. Wassin tentatively suggests the existence of two factor pairs with a different effect, but the data are scanty as yet.

7. The definite bleaching of black to brown is described in the present paper. A single recessive factor may be responsible in the case quoted. This may be identical with the factor described by Wassin.

8. Agouti coloration is described by Wassin, and probably depends upon a single recessive factor which exerts its influence in the presence of certain colour combinations. The white factor appears to be necessary for its exhibition. The very interesting relationship of this factor to the other colour factors has not yet been fully worked out.

VII. ACKNOWLEDGMENTS.

The writers wish to express their sincere thanks to Mr R. H. Roberts, Foxhall, Denbigh, and to Colonel E. J. W. Platt, Gorddinog, Llanfairfechan, who allowed some of their sheep to be crossed with experimental sheep and provided excellent facilities for observation. They are also indebted to Mr R. M. Graves, Wern, Portmadoc, and to Mrs B. A. L. Jervoise, Herriard Park, Basingstoke, for useful information respecting the Black Welsh Mountain breed.

VIII. SUMMARY.

1. Further data are given on the inheritance of the dominant black of the Black Welsh Mountain breed.

2. Results are given that have a bearing on the question of the origin of dominant blacks and the inter-relationship of the dominant blacks of various breeds. It is possible that more than one factor exists that can produce dominant black.

3. It is shown that in the presence of the dominant black factor the factors for badger-face pattern and reversed badger-face pattern cannot be expressed.

4. A brief account is included of some of the modifications of black colour.

5. A very brief summary is given of the present state of knowledge regarding the genetics of coat colour in the sheep.

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STUDIES ON YELLOW-INCONSTANT, A MUTATING CHARACTER OF *PHARBITIS NIL*.

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INTRODUCTION.

A NUMBER of mutating characters have been described in *Pharbitis Nil*, the Japanese morning glory (Imai, 1927 *c*), and of yellow-inconstant, in which green tissues frequently occur in yellow leaves, the writer has already published a preliminary account (Imai, 1927 *a*), while more recently Miyazawa (1929) has also written on this subject. During the past three years I have collected further data now presented in this paper.

THE IDENTIFICATION OF YELLOW-INCONSTANT.

The "Matsushima" variegation, or yellow-inconstant, character was identified by the occurrence of green areas on the yellow leaves. The green tissues, the presence of which is due to a reversional mutation of yellow, cannot, however, be observed on all individuals carrying the yellow-inconstant gene. Owing to this fact, the identification of yellow-inconstant was at times impossible, and it was often erroneously recorded as yellow. In my own strain of yellow-inconstant about 80 per cent. of seedlings were "false yellow," whereas in Miyazawa's mutant strain of yellow-inconstant, the figure was about 24 per cent., the difference being mainly due to the different mutability of the two strains. Under such circumstances, it is difficult to analyse data in which segregation of yellow-inconstant occurs together with that of normal yellow. In 1927, when making observations on yellow-incon-

stant, I called attention to the presence of fine green spots on the yellow leaves, even on plants apparently yellow. Sometimes the green spots cannot be detected on particular leaves of a yellow-inconstant plant, but further observation on the other leaves will reveal its phenotype. In doubtful cases a hand lens or binocular microscope of low power may help in the detection of the spots. If the plants are making a luxuriant growth the identification is easy. The discovery of the phenomenon by which the character can be identified, *i.e.* by the occurrence of fine green spots on the yellow-inconstant leaves, is of much help in its investigation. The fine green spots are due to mutations occurring at a late stage in the cell generations during the ontogeny of leaves. The mutation is so frequent during somatogenesis that every individual of yellow-inconstant can be distinguished by the fine green spots.

THE YELLOW LOCUS.

Yellow-inconstant is recessive to normal green (Imai, 1927 *a*; Miyazawa, 1929), and gives yellow-inconstant in F_1 when it is crossed with yellow (Miyazawa, 1929). In the latter case segregation for yellow-inconstant and yellow occurs in F_2 , with some green mutants. The occurrence of "false yellow" among the yellow-inconstant segregates, however, obscures the genetic relation of yellow-inconstant to yellow, as well as to the green normal. As stated above, we can now identify the yellow-inconstant segregates very accurately by the presence of fine green spots on leaves, and the new data bring out the following points:

(1) Yellow-inconstant appears as recessive, almost exactly in the proportion expected, in F_2 of the cross between yellow-inconstant and normal, as indicated by the data in Table I.

TABLE I.

F₂ from the cross of yellow-inconstant by normal.

Cross	+	<i>y</i> ^e	<i>y</i>	Total
422 × 435	133	40	0	173
190 × 421	302	99	0	401
Total	435	139	0	574

(2) The relation between yellow-inconstant and yellow also is very simple, yellow-inconstant being dominant to yellow. The majority of F_1 plants obtained by crossing yellow-inconstant with yellow showed the former character. Actually I obtained 53 yellow-inconstant F_1 from reciprocal matings, with two green mutants. The results of selfing the yellow-inconstant hybrids are shown in Table II.

TABLE II.

F₂ from the cross of yellow-inconstant by yellow.

Cross	+	y^i	y	Total
421 × 430	1	123	45	169
420 × 34	7	241	77	325
Total	8	364	122	494

The occurrence of a few green plants in this table is due to the mutable property of yellow-inconstant, and they may be here counted as yellow-inconstant, because of their origin from yellow-inconstant. The data show a simple Mendelian segregation, with yellow a recessive to yellow-inconstant.

The F_2 of the green plants obtained among the regular yellow-inconstant F_1 showed segregation for normal and yellow, but not for yellow-inconstant, as indicated in Table III.

TABLE III.

F₂ of green F₁ from the cross of yellow-inconstant by yellow.

Cross	+	y^i	y	Total
421 × 430	156	0	55	211
420 × 34	88	0	28	116
Total	244	0	83	327

The regular F_1 between yellow-inconstant (y^i) and yellow (y) should be y^i/y in its genotype, but the green F_1 is expected to be $+/y$, for the presence of $+$ is due to the transformation of y^i .

As repeatedly proved, yellow is allelomorphic to normal and, as mentioned above, yellow-inconstant also is allelomorphic to normal and to yellow; therefore the three genes, normal, yellow-inconstant and yellow, constitute a triple series of allelomorphs situated at the yellow locus. This locus is in the yellow chromosome (Imai, 1929). Linkage studies also favour the interpretation in terms of multiple allelomorphs (Imai, in press).

Miyazawa's yellow-inconstant appeared as a mutation in his experimental plants, so that his strain is not related to my original strain. From the behaviour of the two strains, especially the numerical proportions of greens and "false yellows," I am inclined to regard them as genetically different.

MUTATING STAGES.

My own yellow-inconstant strain gives 11.5 per cent. of yellow seedlings with green tissues in cotyledons, the others (setting green

mutants aside for the present) being apparently yellow; the actual number observed was 317 of the former and 2430 of the latter. Of the seedlings with green-yellow-mosaic cotyledons, about 51 per cent. (123 among 243) grew up into "false yellows," whereas only about 2 per cent. (5 in 237) of seedlings with yellow cotyledons changed into mosaic, others remaining as "false yellow." The green areas on these mosaic plants can, however, generally be traced back to an embryonic origin. To fix the distribution of green parts on the yellow-inconstant plants, a spiral diagram of the phyllotaxy is convenient (Imai, 1927 *a*). As stated elsewhere (Imai, 1927 *b*), right- and left-handed phyllotaxy occur at random, and consequently both dextrorsal and sinistrorsal diagrams are required. As pointed out by Miyazawa (1929), discontinuity sometimes occurs in the distribution of the mutated cells over the plant body, especially at the junction of the hypocotyl and its upper stem. Furthermore, confusion of cell arrangement in the formation of organs may frequently occur, changing the successive distribution of the mutated cells. The fact that almost all remarkable mosaic plants in the writer's yellow-inconstant strain can be traced back to an embryonic origin for their mutated cells, together with low frequency in the occurrence of massive green tissues of an isolated distribution in the foliage, reveals to us a marked difference of mutability in the different stages of ontogeny. Sometimes small, more rarely large green areas of an isolated distribution occur on the otherwise yellow leaves of the yellow-inconstant; they may have an origin in the cell generations of the post-embryonic somatogenesis, occurring at so early a stage that the propagation of the mutated cells admits of the formation of massive green parts. As already stated, the yellow-inconstant leaves have fine green spots, which probably originate at a late stage during ontogeny. Taking these facts together, we have roughly three periods for the mutating frequency; *i.e.* the mutability is high during embryonic development, low in the post-embryonic somatogenesis, and again high at a late stage of the cell generations in the formation of leaves. The mutating frequency of Miyazawa's yellow-inconstant strain, however, is much higher than in my original strain, and mutations in the general somatogenesis seem to occur more frequently.

TYPES OF BUD VARIATIONS.

The mosaic yellow-inconstant plants sometimes put forth bud variations of green, or chimerical branches. The bud variations may be green, which is solid green without any yellow cells; periclinal (in a narrow

sense) with yellow "skin" and green "core," and the reversal, which also has periclinal (in a broad sense) tissues of green "skin" and yellow "core," as well as sectorial chimeras. Some mosaic plants exhibit manifold bud variations, producing sectorial and periclinal chimeras, green and reversal, and so on.

In the higher plants the tissues of the general plant body are considered to originate from three layers, a problem fully discussed by Chittenden (1927). The original first layer develops into epidermis. Anatomical studies of some periclinal plants show the extent of propagation of the original second layer; that is, for instance, in the stem this layer propagates into outer cortex. The remaining inner region, including the inner cortex and central cylinder, is developed from the original third layer. Therefore, the developmental extension of the three original layers does not entirely coincide with the respective three constituents, dermatogen, periblem and plerome, of the meristem. Noack's theory (1922) is not convincing enough to account for the facts presented by various chimerical plants. Some complicated chimeras were reported and questioned by Chittenden, but the complexity may be induced by recurrent somatic mutations (Imai, 1928).

In the case of yellow-inconstant, it is difficult to determine the colour of the plastids in the guard cells of the epidermis of the leaves, and consequently examination must be confined to the sub-epidermal and more internal regions. The plastid colour of normal green mesophyll is so green that we can readily distinguish it from the yellow under the microscope. The mesophyll of the leaves of *Pharbitis Nil* is composed of one thick layer of palisade, with at times two small cells connected end to end, and several layers of spongy parenchyma arranged irregularly with much intercellular space. The periclinal leaf is composed of yellow sub-epidermal and green inner regions, the former of which includes the palisade and a sub-epidermal region of spongy layer. In the reversal, on the other hand, the palisade and the sub-epidermal region of spongy tissue are green, enclosing a yellow "core." Outwardly, the presence of the yellow "core" in reversal leaves is frequently inconspicuous, and they may be mistakenly classed as green, but careful inspection will reveal its identity. For the periclinal and reversal the tissues of the calyx may give better preparations of the bicoloured arrangement. Miyazawa (1929) seems to doubt the occurrence of the periclinal chimera described by the writer (Imai, 1927 *a*), but he has himself figured a yellow leaf with greenish (light-coloured in his diagram) centre (Miyazawa's Fig. 4, *L*), which is evidently a periclinal composed of yellow "skin" and green

"core." The production of a green part on the periclinal leaf is regarded as due to the protrusion of the inner green tissue through the yellow "skin," a phenomenon frequently observed in the writer's yellow-inconstant as well as in chimerical plants generally.

The tissue development of the embryo is different from the otherwise general somatogenesis. In the early developmental stage of the embryonic tissues, the differentiation of the three original layers has not yet taken place; therefore the production of solid green branches may occur, by chance, when the cells have occupied the "Anlage" of buds. In the later somatogenesis, however, this occurrence must be rare, because of the differentiation of the three original layers. Let us attempt to consider more definitely the mechanism of the so-called bud variations, which occurred after the differentiation of the three layers of different origin. Suppose a mutation occurs in an epidermal cell and the mutated cell becomes propagated in the epidermal layer covering the growing point of a bud; in such a case, we shall have a bud variation with the mutant epidermis covering the prototypic inner constituents. If this occurs in the second layer, a bud variation with the mutant sub-epidermal region, covering the prototypic "core" and being covered by the unaltered epidermis, will occur; and if in the third layer, a bud variation with the mutant "core" covered by the prototypic "skin" may be produced. These three bud variations are of primary types, but they may sometimes be transformed into the other different types during tissue development. It is well known that periclinal or reversal plants at times give branches with homogeneous tissues; and also that they rarely do so with inverse arrangement of heterogeneous tissues, from periclinal to reversal or *vice versa*. This does not denote the occurrence of a new mutation, but is due to confusion in the distribution of cells or tissues during development. Because of this confusion, a bud with a growing point composed of the second and third original layers casting off the first, or composed of the first layer excluding the second and third, or others, may occur. Under such conditions, secondary types of bud variations may be produced. When the given bud variations are indifferent to the embryonic origination, the possibility of those with homogeneous tissues must be rare, since this production may be induced by three, rather infrequently occurring factors: firstly, the mutation; secondly, confusion in the arrangement of tissues; and thirdly, the resultant situation of the cell layers on the growing points of buds, taking place successively one after the other. From this point of view the so-called bud variations observed in various plants must be critically

examined. East (1917) drew attention to the possibility of a connection between periclinal chimeras and bud variations, and suggested a periclinal explanation for the nectarine. Asseyeva (1927) found the bud variations of the potato to be chimeras, and she isolated the prototypic forms from the mutant potato tubers by the removal of the "eyes." When the mutation can be traced back to an embryonic origin, however, bud variations homogeneous for the mutant tissues may be produced. Actually, green bud variations of this sort were observed at times in yellow-inconstant.

THE OFFSPRING OF CHIMERICAL PLANTS.

An examination of the offspring of bud variations related to yellow-inconstant has already been made (Imai, 1927 a), proving their correspondence with the genetical constitution of the sub-epidermal region of the mother branches. The later data confirm this. The offspring of the prototypic and varied branches of mosaic plants are shown in Table IV.

TABLE IV.

Offspring of chimerical yellow-inconstant plants.

Plant	Type of branch	Green	Green variegated	False yellow	Total
3-1	False yellow	3	4	65	72
	Sectorial	50	5	52	107
	Periclinal	1	3	26	30
3-15	Reversal	88	4	21	113
	Green	31	1	9	41
5-1	Periclinal	1	5	90	96
5-3	False yellow	0	0	15	15
	Reversal	35	2	11	48
	Green	27	0	8	35
6-1	False yellow	1	5	56	62
	Sectorial	40	4	77	121
	Green	44	1	10	55
6-6	False yellow	0	0	2	2
	Green	89	3	26	118
8-1	Periclinal	0	2	27	29
10-3	False yellow	1	8	155	164
	Periclinal	1	2	34	37
9	Chimerical plants	299	45	298	642

The results correspond fairly with the genotypes of the sub-epidermal tissues, from which the following generation originated through fertilization.

GREEN MUTANTS.

In the writer's strain, when only the offspring of the so-called false yellow, on which no massive green areas were discernible by close examination, are taken into account, the production of the green, green variegated and false yellow seedlings is 1.9 per cent., 11.3 per cent. and 86.8 per cent. (52, 317 and 2430 in number) respectively. When it is considered that some of the greens thus appearing might have been produced by gametic mutation, in addition to those which have had a chance to develop into green cotyledonous seedlings by the overcoming of, or the monopolistic distribution of, the green cells mutated in the early stage of the embryonic ontogeny, the production of the green seedlings is not as high as we expect it to be, compared to that of the mosaic. Hence the green mutants thus making their appearance should not be regarded as necessarily originating by mutation at gametogenesis. Furthermore, the occurrence of fine green spots on the otherwise yellow leaves may give the same corresponding distribution of the genetically green cells in the germinal tissues. If this is the case, a few green mutants may be produced in the following generation, with a somatic origin, but occurring in the mother sporophyte. Hence the green mutants cannot here be classified in respect of their origin. In Miyazawa's yellow-inconstant strain, the proportion of green, green variegated and false yellow plants is 14.0 per cent., 61.6 per cent. and 24.4 per cent. respectively. The proportion borne by the green to the green variegated is similar to that in the writer's strain, suggesting a type of mutation in the two cases similar but differing in frequency.

TABLE V.

Offspring of green mutants in the yellow-inconstant strain.

Plant	Green	Green variegated	False yellow	Total
2	32	3	8	43
3	19	1	5	25
48	96	5	29	130
72	92	2	20	114
13 (1)	18	0	4	22
(8)	48	3	14	65
18 (3)	30	1	8	39
(5)	14	1	4	19
Total	349	16	92	457

The green mutants appearing in yellow-inconstant have been proved to be heterozygous for yellow-inconstant. Fresh data favouring this are given in Table V.

The proportion of green segregates is 76.4 per cent., which surplus, however, is not remarkable. In Miyazawa's strain it amounts to 83.5 per cent., owing to the high mutability of his yellow-inconstant strain.

SUMMARY.

1. The seedlings or plants carrying the gene "yellow-inconstant" sometimes are characterised by green variegated areas on the otherwise yellow foliage. The presence of green tissues is due to reversional mutations occurring in somatogenesis. The majority of the offspring are apparently yellow, without any distinct areas of green. Nevertheless close examination of the leaves of yellow-inconstant, and even of false yellow, reveals fine green spots, which are due to vegetative mutations occurring at a late stage of cell division in the ontogeny of the leaves.

2. Yellow-inconstant is dominant to yellow, and recessive to normal green. The three genes, normal, yellow-inconstant and yellow, constitute triple allelomorphs.

3. The frequency of mutations of yellow-inconstant to normal green on the yellow-inconstant sporophyte is not uniform throughout its growth, but can be roughly divided into three periods; for the mutability is high in the embryonic development, low in the post-embryonic somatogenesis, and again high at a late stage of cell division in the ontogeny of the leaves.

4. Yellow-inconstant sometimes puts forth bud variations of solid green, as well as sectorial and periclinal chimeras. In almost all of the remarkable mosaic plants of yellow-inconstant in the writer's strain mutated green cells can be traced back to an embryonic origin.

5. The production of solid green bud variations may not be rare, when mutation occurs during embryonic development. In the later somatogenesis, however, this occurrence must be rare, because of the differentiation of the three original layers. The bud variations induced by such an origination may be composed of heterogeneous tissues, with prototypic parts enclosed or being enclosed by the mutant parts, but not of homogeneous mutant tissues.

6. The genetic aspect of the offspring of various types of bud variations in the yellow-inconstant such as green, periclinal, reversal and sectorial, corresponds fairly to the respective genotypes of the sub-epidermal tissues of the mother branches.

7. The green mutants appearing in the yellow-inconstant are heterozygous for yellow-inconstant, giving nearly simple Mendelian segregation in their offspring. The deficit of the recessive yellow-inconstant segregates

is not much in the writer's strain, whereas it is remarkable in Miyazawa's strain, owing to its high mutability.

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CYTOLOGICAL STUDIES OF SOME HYBRIDS OF *AEGILOPS* SP. \times WHEATS, AND OF SOME HYBRIDS BETWEEN DIFFERENT SPECIES OF *AEGILOPS*.

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(With 297 Text-figures.)

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INTRODUCTION.

DURING the last few years investigations of the cytology of *Aegilops* \times wheat hybrids have been made by Bleier(1), Gaines and Aase(4), Jenkin(6), Kagawa(7) and Sax(11). The researches were chiefly concerned with an examination of the meiotic phase in pollen mother cells of a small number of hybrids only, the observations usually beginning with the metaphase of the heterotype division.

In a previous communication(9) I gave an account of some hybrids

of *Aegilops ovata* crossed with varieties of the tetraploid wheats, *T. dicoccoides*, *T. dicoccum* and *T. durum*, and with varieties of the hexaploid race *T. vulgare*, attention being given to both the morphological and the cytological results.

More recently I have extended the scope of the investigations, and thirty-three hybrids of four species of *Aegilops* with diploid, tetraploid and hexaploid wheats have been studied, as well as hybrids of the four species of *Aegilops* crossed among themselves.

It was thought that a comparative study of the morphological and cytological features of a carefully planned series of hybrids would provide a wider and clearer view of the relationships of the plants concerned, than the examination of single casually selected crosses, and at the same time would advance our knowledge of the morphology and cytology of hybrids in general.

The present communication is concerned only with the cytological results; it is hoped to deal later with the morphology of the plants and their hybrids.

MATERIAL AND METHODS.

The range of the investigations may be briefly indicated as follows:

A. Hybrids of *Aegilops* sp. \times wheats. All sterile.

Series I. *Aegilops ovata* L. $\varnothing \times$ wheats σ .

II. *Aegilops cylindrica* Host $\varnothing \times$ wheats σ .

III. *Aegilops triuncialis* L. $\varnothing \times$ wheats σ .

IV. *Aegilops ventricosa* Tausch. $\varnothing \times$ wheats σ .

B. Fertile hybrid.

Aegilops ovata L. $\varnothing \times$ *T. turgidum* L.

var. *iodurum* σ .

C. Hybrids of species of *Aegilops*. All sterile.

1. *A. cylindrica* Host $\varnothing \times$ *A. ovata* σ .

2. *A. triuncialis* L. $\varnothing \times$ *A. cylindrica* Host σ .

3. *A. ovata* L. $\varnothing \times$ *A. ventricosa* Tausch. σ .

4. *A. cylindrica* Host $\varnothing \times$ *A. ventricosa* Tausch. σ .

Pollination was carried out both in the open and under glass, and the hybrid F_1 generation was grown in the greenhouse, the seed being sown late in January.

Cytological investigations were made of the meiotic phase in the pollen mother cells of all the hybrids.

The method used was similar to that described in a former communication (9), but Carnoy's solution was employed for the permanent preparations and gave exceedingly good results, even in the very fine spireme stage of very early prophase, if allowed to act for not less than half an hour, followed by careful washing with absolute alcohol to remove all traces of acetic acid.

Owing to the number of hybrids and the time needed to examine thoroughly such a large amount of fixed material, it was decided to pay chief attention to the stages of meiosis after the formation of the synzytic knot. In a few instances, however, pollen mother cells in earlier prophase were fixed, and these proved of much service in elucidating some of the peculiar cytological features found in these hybrids.

Longitudinal and transverse sections were cut of a thickness of 12-14 μ , and stained with Heidenhain's iron-alum-haematoxylin.

SERIES I. *AEGILOPS OVATA* L. ♀ × WHEATS ♂.

The following hybrids in this series were studied:

(a) Triploid hybrid.

$n = 14$

$n = 7$

A. ovata L. ♀ × *T. monococcum* L. (Einkorn) ♂.

(b) Tetraploid hybrids.

$n = 14$

$n = 14$

A. ovata L. ♀ × *T. dicoccoides* Körn. (Wild Emmer.)

var. *Kotschyianum*. ♂.

var. *spontaneonigrum*. ♂.

× *T. dicoccum* Schüb. (Emmer.)

var. *Ajar*. ♂.

var. *persicum*. ♂. (Black Persian.)

var. *ethiopicum*. ♂.

× $\frac{1}{2}$ *T. polonicum* L. ♂.

× *T. turgidum* L. (Rivet Wheat.)

var. *mirabile*. ♂. (Miracle.)

× *T. durum* Desf. (Macaroni Wheat.)

var. *affine*. ♂.

(c) Pentaploid hybrids.

$n = 14$

$n = 21$

A. ovata L. ♀ × *T. compactum* Host. (Club Wheat.)

var. *creticum*. ♂.

A. ovata L. × *T. sphaerococcum* Perciv. (Indian Dwarf Wheat.)
var. *tumidum*. ♂.

× *T. Spelta* L. (Dinkel.)
var. *coeruleum*. ♂.

× *T. vulgare* Host. (Bread Wheat.)
var. *albidum*. ♂. (Starling.)

T. vulgare Host ♀ × *A. ovata* L. ♂.
var. *graecum* ♀

(d) Fertile hybrid.

$$n = 14$$
$$n = 14$$

A. ovata L. ♀ × *T. turgidum* L. (Rivet Wheat.)
var. *iodurum*. ♂. (Poulard d'Australie.)

(a) Triploid hybrid.

Aegilops ovata L. ♀ × *Triticum monococcum* L. ♂.

In the youngest pollen mother cells observed, the nuclear cavity contains a deeply staining nucleolus and thin elongated threads, some of which appear in places to coalesce longitudinally.

Later, the threads become thickened and separate chromosomes are seen, diploid in number in some of the cells. Condensation and contraction of the nuclear contents continues until a dense synizetic knot is seen, in which the outlines of the chromosomes are completely lost, with the exception of one or two which are sometimes not entirely absorbed in it (Figs. 1-4).

Up to this stage the nucleolus and nuclear membrane are clearly distinguished. In the next stage the nuclear membrane has gone and the deeply stained knot appears imbedded in dense cytoplasm. The knot now begins to open out; a bipolar spindle arises and the diploid number of short, thick chromosomes (21) can be counted. In some of the cells only separate univalents are seen scattered irregularly in the cytoplasm, but in most cells from 1 to 5 bivalents are found, the individual chromosomes of each pair being joined end to end by a fine thread. Such pairs take a central position on the spindle, with the univalents distributed around in no definite order, though sometimes from 3 to 7 of them encircle the bivalents in the equatorial zone of the cell. At a slightly later stage of the heterotype division two polar groups of chromosomes are seen, usually 4 to 8 in each, together with a more or less central group consisting of a variable number of lagging univalents and

the components of any of the bivalents which have not completely separated (Figs. 5-9).

In the metaphase and anaphase of the heterotype division many of the univalents are longitudinally split (Fig. 10), this occurring soonest in cells in which there are no bivalents. Usually the halves of the split univalents remain united and travel together to the same pole, but in some cases the halves of the univalents which lie in the equatorial zone separate from each other and travel to opposite poles (Fig. 11).

In the telophase of the first division, undivided univalents may occasionally be observed at each pole, together with univalents showing the homotypic split, and monad chromosomes or halves of completely divided univalents which have travelled to opposite poles after separation on the equatorial plate as previously noted. The number of univalents or their divided equivalents which enter the daughter cells of the heterotype dyad varies between 8 and 13: sometimes 10 may be seen at one pole and 11 at the other. In all such cases the chromosomes content of the two cells is necessarily different, but owing to the fact that at this stage of meiosis a number of univalents undergo the homotypic division with separation of the resulting monads, it is possible that the two cells of the heterotype dyad may receive exactly the same number of chromosomes, and observations on the anaphases of the succeeding homotypic division show that this occurs, for halves of dyads are sometimes seen containing 21 monad chromosomes, the equivalent of $10\frac{1}{2}$ univalents (Fig. 15).

At the completion of the heterotype division most of the chromosomes become incorporated in the telophase nuclei, very few lagging and becoming isolated in the cytoplasm.

After a short interkinesis the homotypic division commences, the metaphase of which is more regular than the metaphase of the preceding division, all the chromosomes often forming a more or less typical equatorial plate (Fig. 14). The movement from the metaphase, however, is irregular and in the anaphase three groups of chromosomes are nearly always found, one at each pole, with a lagging third group in the centre of the spindle (Figs. 15, 17). The number in the central group varies from 2 or 3 to 10 or more. As already noted above, 21 monad chromosomes can sometimes be counted in one or both of the two dividing cells (Fig. 15), but the number is usually very different from this owing to uneven distribution in the heterotype division and the presence of undivided univalents some of which do not divide at any stage of meiosis (Figs. 18, 20).

The anaphase of the homotype division is often very irregular with many lagging chromosomes, and although a large proportion of the final divisions of the pollen mother cells result in the formation of tetrads of four cells of similar size, few if any of the nuclei in the latter are normal; micro-nuclei and isolated single chromosomes are nearly always present in the cytoplasm of the cells (Figs. 18, 19). In cases where a tripolar spindle is formed in the heterotype division the mother cells at the homotype division give rise to 6 microspores of variable size with irregular nuclei, some of them when fully developed being totally empty or containing only cytoplasm without visible chromatin (Figs. 20, 21).

In one loculus of an anther were observed several cells containing about 35 univalent chromosomes (Figs. 22-24); these appear to have arisen through cytomixis between adjacent cells as indicated in Fig. 24 taken from the same loculus.

(b) Tetraploid hybrids.

A. ovata L. ♀ \times *T. dicoccoides*.

var. *kotschyannum* ♂.

var. *spontaneonigrum* ♂.

\times *T. dicoccum*.

var. *Ajar* ♂.

var. *ethiopicum* ♂.

A. ovata ♀ \times *T. dicoccum* var. *Ajar* ♂.

In this hybrid the leptotene stage of the microspore mother cells was observed. At this time the nuclear cavity is permeated by an extremely fine unsplit spireme which is not continuous, but broken into threads of variable length, some of which are bent into loops. Later, the threads coalesce longitudinally, after which they become much shorter and thicker, ultimately contracting into a dense synizetic knot in which the outlines of the chromosomes are obliterated although portions of short, thick loops may sometimes be seen projecting from it (Figs. 25-28).

Hitherto the nucleolus is conspicuous, and the nuclear membrane clearly seen. The latter, however, soon disappears, and from the knot which now lies imbedded in the dense cytoplasm, the short, thick chromosomes begin to emerge. The thickening of the spireme, transverse segmentation into short chromosomes, condensation into a dense knot, and unfolding of the latter with the separation of short thick univalents, takes place rapidly, for all these stages are sometimes present in the same loculus of an anther.

The diploid number of chromosomes in these hybrids is 28 as expected, 14 being derived from each parent; these figures were determined from somatic cells as well as from pollen mother cells.

The dense synizetic knot opens out directly into separate chromosomes, a bipolar spindle appearing at the same time. Diakinesis with paired chromosomes is not seen, nor is a typical metaphase ever found with all the chromosomes arranged on a definite equatorial plate. In the metaphase 3-7 univalents are usually located near the opposite poles of the cell, the rest being scattered irregularly over a bipolar or tripolar spindle, with one or two often in the cytoplasm of the cell away from the spindle. In most cells there are only univalents, or univalents and a single end-to-end bivalent, but occasionally 2 or 3 bivalents are seen arranged on the central fibres of the spindle, 3 being the maximum number observed (Figs. 29-32).

The components of the bivalents behave normally, separating to opposite poles, but the movement of the univalents is erratic, and some of them lag for a time in the equatorial zone. The numbers which finally collect at the poles vary from 12 to 18, though 14 at each pole is not unfrequent in many cells.

Before the completion of the anaphase of the heterotype division most of the univalents become longitudinally split (Figs. 33-35), but a few sometimes remain undivided for a considerable time, one or two occasionally being found isolated in the cytoplasm of the microspores throughout the whole meiotic phase (Figs. 39, 41).

In the majority of cells the later anaphases show two groups of univalents, single or divided, at the poles of the spindle, and, more centrally placed, two groups of monad chromosomes travelling to opposite poles, the latter being the halves of divided univalents which have lagged behind the rest. Thus, at the poles, in this stage may sometimes be seen undivided univalents, split univalents, and monad chromosomes or halves of divided univalents (Figs. 34, 35).

In mother cells in which 14 chromosomes travel to each pole, the heterotype division appears to result in the formation of a typical dyad with normal nuclei, but in most cases the number of chromosomes which find their way into the two cells of the dyad varies considerably, and the nuclei of the latter are usually of different size and composition; occasionally one or more undivided univalents are observed in the cytoplasm of the divided cell outside any nucleus.

Irregular tripolar distribution of univalents in the heterotype division sometimes occurs as in Fig. 40, this being followed by the formation of

a triad, in each cell of which is usually a different number of chromosomes.

In the succeeding homotypic division there is a differential movement of the chromosomes to the poles of the spindle similar to that of the univalents in the heterotype division, which leads to a figure of two groups at opposite poles, with one or two groups lagging in the equatorial zone (Figs. 37, 38). The final division of the dyad cells and distribution of chromosomes is, however, more irregular than in the heterotype division, the number of chromosomes entering the telophase nuclei of the tetrads being very variable. Some arrive at the poles of the spindle too late to be included in the nuclei, and remain isolated in the cytoplasm. Moreover, while some of the mother cells are divided into the normal 4-celled tetrad, from many of them 5 to 6 microspores of variable size and chromosome content are produced, the former number when a tripolar arrangement is established in one of the two cells produced by the first division, 6 microspores resulting from the final division of a mother cell in which a tripolar spindle is formed in the first or heterotype division (Figs. 39-41).

A. ovata ♀ \times *T. polonicum*.
 \times *T. turgidum*.
 var. *mirabile* ♂.
 \times *T. durum*.
 var. *affine* ♂.

In all these hybrids the cytological details are similar to those described above. In 60-70 per cent. of the cells 28 scattered univalents are present in the heterotype division; 20-30 per cent. have 1 end-to-end bivalent, only a small percentage having 2 (Figs. 42, 43, 46, 47). The number of univalents or their divided equivalents which move to the poles in this division, varies as in the previously described hybrids, but the equal division of 14 to each pole is perhaps more frequent. There is also similar longitudinal division of the univalents at this stage of meiosis (Figs. 44, 45, 48).

In the homotypic division the chromosomes in the metaphase are generally arranged on a regular equatorial plate, but in all cases the subsequent movement and distribution in the anaphase is irregular, showing a group of lagging chromosomes in the equatorial zone with a group at each pole (Fig. 49).

The final division results in the production of 4-celled tetrads, though some of the cells are not infrequently of different sizes; in addition to

4-celled tetrads some of the mother cells become divided into 5 or 6 cells.

A. ovata ♀ × *T. dicoccum* Schüb.

var. *persicum* Perciv. ♂. (Black Persian.)

(= *T. persicum* Vav.)

In a few of the mother cells of this hybrid, the 28 univalents which emerge from the synizetic knot are scattered irregularly as in the hybrids previously described (Figs. 50–53), but in a large number of the cells there is a marked tendency for all or most of the univalents to collect and form a good equatorial plate in the metaphase of the heterotype division (Figs. 54–56), a feature absent in hybrids of this species of *Aegilops* with other races of wheat.

Single end-to-end bivalents are occasionally seen (Fig. 54), but are rare; one or two isolated lagging chromosomes are sometimes observed in the cytoplasm off the spindle.

As in the other hybrids described, longitudinal division of the univalents takes place in the heterotype division, even in those cells in which they are on the equatorial plate (Figs. 56, 57); lagging of the divided chromosomes, and the formation of dyads with different numbers of chromosomes in each of the two cells are common cytological features.

The homotype division is also quite similar to that of the other tetraploid hybrids examined.

(c) Pentaploid hybrids.

A. ovata L. ♀ × *T. Spelta* var. *coeruleum* ♂.

In the early prophase are seen comparatively short chromosomes which thicken and crowd into a dense synizetic knot (Figs. 60–62), the nucleolus and nuclear membrane disappearing as the change proceeds. From the compact knot which lies imbedded in the dense cytoplasm, the chromosomes unfold, and the diploid number (35) of univalents is clearly distinguished.

As in the heterotype division of the triploid and tetraploid hybrids, typical metaphases in which all the chromosomes appear on an equatorial plate are not seen, the 35 univalents being scattered irregularly throughout the cell, a few often in the cytoplasm away from the spindle. In the majority of the cells only separate univalents are observed, but in a few 1 to 3 end-to-end bivalents are present, as in the previously described hybrids (Figs. 64–66).

Movement of the chromosomes into the anaphase position proceeds irregularly, and most of them undergo longitudinal division at this time, but only the halves of those lying in the equatorial region of the spindle separate from each other and travel to the opposite poles. One or two univalents sometimes remain undivided both in this and the succeeding homotypic division (Fig. 68).

Owing to the irregular distribution of the chromosomes in the anaphase of the heterotype division many more may be collected at one pole than the other, and the nuclei of the two cells of the dyad often vary considerably in chromosome content. Tripolar spindles are not infrequent at this stage, and the first division of the pollen mother cell may result in the formation of a triad instead of the normal dyad.

In the homotype division there is a differential movement of the chromosomes as in the heterotype division, variable numbers lagging behind in the equatorial zone (Figs. 70-72).

In both the heterotype and homotype divisions chromosomes are observed off the spindles of the dividing cells, and these, with some which lag for a time in the equatorial region, are not included in the nuclei formed at the poles, but are either left as isolated chromosomes, or combine to form dwarf nuclei in the cytoplasm of the microspores.

While many of the mother cells give rise to four microspores, a large number are divided into 4 to 6 cells of unequal sizes; some of them are very small and cut off the cytoplasm of the divided cell without reference to the position of the spindle (Figs. 73, 74). A mother cell in which a tripolar spindle appears in the heterotype division, often gives rise to a hexad of cells all similar in size at the final homotype division (Figs. 75, 76).

<i>A. ovata</i> ♀	\times <i>T. compactum</i> .
	var. <i>creticum</i> ♂.
	\times <i>T. vulgare</i> .
	var. <i>albidum</i> ♂.
	\times <i>T. sphaerococcum</i> .
	var. <i>tumidum</i> ♂.
<i>T. vulgare</i> ♀	\times <i>A. ovata</i> ♂.
var. <i>graecum</i>	

The cytology of all these hybrids is closely similar to that described above (Figs. 77-93). In the hybrid with *T. sphaerococcum* bivalents are, however, more frequent; and the maximum number observed was 4 instead of 2 or 3 as in the other crosses.

It is interesting to note that in this particular hybrid in which the maternal parent was wheat, and not *Aegilops* as in the rest, no differences in the cytological details were observed, the distribution of the chromosomes being apparently independent of the nature of the cytoplasm of the mother.

Comparison of the results of these investigations reveals exact similarity in much of the cytological detail in the meiotic phase of the triploid, tetraploid and pentaploid hybrids of *Aegilops ovata* with different races of wheat.

Unfortunately in the majority of cases the earliest prophases were not examined and fixed; in the few, however, which were preserved, the finest spireme consists of discontinuous unsplit threads. Later these coalesce longitudinally into thicker threads from which arise separate chromosomes, diploid in number. This stage is followed by the formation of a dense synizetic knot, which opens out into what may be termed the early metaphase of the heterotype division, the chromosomes taking their place on the spindle which appears, diakinesis being cut out of the phase.

In the majority of pollen mother cells only separate univalents are found at this stage, diploid in number; but in some cells are seen bivalents consisting of short thick univalents, each pair being joined end to end by a fine thread.

In the hybrid *A. ovata* \times *T. monococcum* the maximum number of bivalents is 5; in the hybrids *A. ovata* \times wheats of the Emmer series the maximum is 3, the maximum number being also 3 in the hybrids with varieties of the Bread Wheat series, except in the hybrid *A. ovata* \times *T. sphaerococcum*, in which the maximum of bivalents is 4, although cells are numerous in which none or only 1 to 3 are observed.

In the early metaphase of the heterotype division the univalents are always scattered irregularly over the spindle, with a few sometimes in the surrounding cytoplasm; bivalents when present take their place on the central fibres of the spindle, often with many of the univalents distributed around them in the equatorial zone. No clearly marked differences in the size or form have been discovered between the chromosomes of *A. ovata* and the wheats, and the two cannot be distinguished in the dividing cells. Moreover, the position and movement of the scattered univalents is too erratic, and the observations too few to allow of reliable conclusions regarding the distribution of the chromosomes of the two parents in the meiotic divisions. Nor is it possible to decide whether the end-to-end bivalents consist of homologues from the different parents,

or are composed of united chromosomes from the same parent. It may, however, be noted that in the early metaphases the combined number of univalents at the two poles is not unfrequently 14 or near this figure, the number left in the equatorial zone approximating to 7 in the triploid hybrid, 14 in the tetraploid, and 21 in the pentaploid hybrid respectively; this suggests that the 14 chromosomes lying near or first reaching the poles belong to the *Aegilops* parent, the rest, 7, 14 and 21 respectively, being chromosomes of the wheats.

The 7 chromosomes frequently left in the equatorial region in the anaphase of the heterotype division of the triploid hybrid may of course be regarded as belonging to the *Aegilops*, the 7 chromosomes of the wheat parent travelling to the poles with 7 homologues from the *Aegilops* parent, the remaining 7 of the 14 belonging to the latter remaining in the equatorial region.

On the assumption, however, that they are loosely paired homologous chromosomes belonging to the two parents which move to the poles, no "laggers" would be expected where both parents contribute an equal number of chromosomes, nevertheless a central lagging group, often 14 in number, is frequent in all the tetraploid hybrids examined. Similarly, only 7 chromosomes lagging in the equatorial region with 14 homologues at each pole would be expected in the pentaploid hybrids, instead of which a larger number is usually seen in the equatorial zone, with fewer at the poles in most of the earliest stages of the metaphases of the heterotype division. I am inclined to the view that the groups which first reach the poles consist of chromosomes of the *Aegilops* mother parent, and therefore intimately adapted to the environment of the cell, the lagging group being pollen parent wheat chromosomes and aliens in the cytoplasm.

A striking feature of all these hybrids is the longitudinal split in almost all the univalents in the anaphases of the heterotype division. Where the univalents reach the poles of the spindle, their halves remain together and enter the same nucleus; but those of the univalents lying in the equatorial zone separate to opposite poles and enter different nuclei.

Sometimes the number of chromosomes included in the nuclei of the two cells of the dyad is approximately the same but owing to their irregular movement towards the poles of the mother cell, one-half of the divided cell frequently receives more than the other, and isolated and slow moving chromosomes often fail to become included in either nucleus.

In the succeeding homotypic division of all these hybrids there is a lagging group of chromosomes in the equatorial zone similar to that in the heterotype division, with a corresponding variation in the number entering the nuclei of the tetrads. Not unfrequently 4 microspores of similar size and form are produced from each mother cell, but their chromosome content is variable. Many mother cells are divided into 4 to 6 daughter cells of different sizes and chromosome content. Twin microspores are also formed occasionally owing to imperfect cleavage of the cytoplasm, and small portions of cytoplasm containing 1 or 2 chromosomes are sometimes cut off and ultimately give rise to minute microspores.

SERIES II. *AEGILOPS CYLINDRICA* Host ♀ × Wheats ♂.

The following hybrids in this series were studied:

(a) Tetraploid hybrids.

$n = 14$

$n = 14$

A. cylindrica Host ♀ × *T. dicoccoides* Körn.

var. *rubrivillosum* ♂.

× *T. dicoccum* Schüb.

var. *farrum* ♂. (France.)

× *T. polonicum* L. ♂.

× *T. turgidum* L.

var. *iodurum* ♂. (Pétianelle noire de Nice.)

(b) Pentaploid hybrids.

$n = 14$

$n = 21$

A. cylindrica Host ♀. × *T. compactum* Host.

var. *rubriceps* ♂.

× *T. vulgare* Host.

var. *erythrospermum* ♂. (Asia Minor.)

var. *milturum* ♂.

× *T. Spelta* L.

var. *Duhamelianum* ♂. (Swiss.)

(a) Tetraploid hybrids.

A. cylindrica ♀ × *T. polonicum* ♂.

In the early prophase of the heterotype division, the spireme consists of thin, unsplit, discontinuous threads, which coalesce longitudinally and frequently become bent into loops later. Thickening and shortening of

the threads follow, and soon separate chromosomes are seen, some of them being longer than the rest (Figs. 94-96). There is further condensation, with the ultimate formation of a dense synizetic knot, the nucleolus and nuclear membrane disappearing when the knot has reached the most compact state (Figs. 97, 98).

Synizesis is very soon followed by the loosening of the knot with the appearance of clearly defined, short, thick chromosomes and the early metaphase of the heterotype division. A few mother cells are sometimes found containing the diploid number (28) of separate univalents; but in the majority there are present from 1 to 4 bivalents, the components of which are united end to end.

The bivalents arrange themselves on the central fibres of the spindle, the univalents being scattered irregularly in the cell with 5 to 9 at opposite poles, the rest being distributed in the equatorial zone (Figs. 99, 100).

The components of the bivalents separate normally in anaphase, the halves going to opposite poles, but there is random distribution of the univalents. In some cases, 14 of the latter travel to each pole, but usually 12 to 15 find their way to one pole, the complement travelling to the other.

At this stage the univalents are homotypically split (Fig. 101), with the exception of an occasional one or two remaining undivided. Only the monads or halves of the univalents which are found in the equatorial zone when their division occurs, separate and move to opposite poles.

On account of the irregular distribution of the chromosomes in the anaphase of the heterotype division, the two nuclei of the dyad which arises from the division are of variable chromosome content, and very often the remains of lagging chromosomes which have failed to become incorporated in the telophases of the nuclei are found in the cytoplasm of the divided cell (Fig. 102).

In the homotype division which follows (Figs. 104, 105), the chromosomes are usually arranged on a definite regular equatorial plate, but in the subsequent anaphases irregular movement occurs, and the number of chromosomes distributed to the poles of the homotypic spindle and entering the telophase nuclei vary considerably. Although the great majority of the mother cells are ultimately divided into apparently typical 4-celled tetrads, the chromosome content of the several cells is variable.

A. cylindrica ♀ \times *T. dicoccoides*.

var. *rubrivillosum* ♂.

A. cylindrica ♀ × *T. dicoccum*.

var. *farrum* ♂.

× *T. turgidum*.

var. *iodurum* ♂.

In all these hybrids the cytological details are exactly similar to those of the tetraploid just described (Figs. 106–122).

At the stage of prophase of the heterotype division immediately before the formation of the dense synizetic knot (Figs. 96, 106), the chromosomes are often clearly defined and frequently 24 in number. In many of the cells, some of the chromosomes are approximately double the length of the rest, as in Fig. 106, where 4 are seen; these are, presumably, end-to-end bivalents.

(b) Pentaploid hybrids.

A. cylindrica ♀ × *T. compactum* var. *rubriceps* ♂.

In the earliest prophase observed the spireme is segmented into chromosomes of different lengths, of which a few are apparently bivalents, being longer than the rest and often curved like a horseshoe or bent completely round in the form of oval loops (Fig. 123). Later, both univalents and bivalents are more clearly recognised, the components of the latter being associated in a side-by-side position (Fig. 124). The nucleus now has the appearance of diakinesis, but contraction into a dense synizetic knot follows this stage, the knot opening out into the heterotype metaphase (Figs. 125–130).

At this stage the component chromosomes of the bivalents are completely united or fused into single structures which are, however, readily distinguished from univalents by their greater width, and usually blunt, divided ends. In metaphase polar view they are conspicuous and easily counted, since they occupy the centre of the equatorial plate with a number of univalents distributed round them (Fig. 128).

In the majority of cells the number of bivalents is 7. Around them are scattered 21 univalents, 5 to 12 of which are often near the poles of the spindle, the distribution being best seen in profile views of the dividing cell (Figs. 129, 130). Rarely are all in one equatorial plane.

The behaviour of the bivalents is normal, the halves separating from each other in anaphase and travelling to opposite poles in the usual manner, joining the univalents which are located from the first in the polar region. A variable number of univalents—sometimes as many as 13 or 14—remains for a time in the equatorial zone.

In the heterotype division all the chromosomes except one or two, which sometimes lie off the spindle, divide homotypically. Some, already split, travel in this condition to the poles, but the halves of those which divide while near the equatorial zone separate from each other and move to opposite poles; so that the chromosomes at the heterotype poles may consist of undivided and longitudinally divided univalents, as well as of monads, or single halves of univalents.

The number of chromosomes distributed to opposite poles in the heterotype division varies from 5 to 16, but in a large number of cells 10 unpaired univalents find their way to one pole and 11 to the other, 7 being added to these numbers later from the dividing bivalents. A few univalents, or their split halves, often lag behind and fail to become incorporated in the telophase nuclei of the dyad.

In the second or homotype division of the mother cell, all the chromosomes are frequently collected on a clearly defined equatorial plate, but their movement from this position is irregular, and in anaphase a small number lag behind the rest (Figs. 133, 134), these ultimately appearing as isolated chromatic material, or dwarf nuclei, in the microspores. Most of the mother cells give rise to 4-celled tetrads, but a few are divided into 5 or 6 cells of different sizes. No matter, however, how they are divided the distribution of the chromosomes is irregular, and comparatively few nuclei are unaccompanied by micronuclei or isolated chromosomes in the cytoplasm.

A. cylindrica ♀ \times *T. vulgare*.

var. *erythrospermum* ♂.

var. *milturum* ♂.

Good examples of prophases of the heterotype division of these hybrids were obtained. In the earliest stage the nuclear cavity contains a single large nucleolus, and a very fine leptotene spireme which consists of thin unsplit threads of different lengths (Fig. 126). Later the filaments are double, the doubling being brought about by the approximation of the fine threads (Fig. 167). At a somewhat later stage, shorter, thicker chromosomes appear, some of which still show their double nature (Fig. 138). Further condensation occurs with the formation of a dense synizetic knot, the disappearance of the nucleolus, and the nuclear membrane as in the previous hybrids, in some of the cells single chromosomes, oval bivalents and thick loops being seen (Fig. 139). On opening out of the knot, univalents and 7 bivalents appear, the latter arranged

at the centre of the spindle with the univalents scattered irregularly (Figs. 140-142).

The heterotype metaphase and anaphase are like those of the hybrid previously described, as are also the second division and microspore formation.

The results are in agreement with the researches of Sax⁽¹¹⁾ and Kagawa⁽⁷⁾ who investigated the cytology of a similar hybrid. Neither of these authors, however, refers to the peculiar features of the early prophase and synizesis.

A. cylindrica ♀ × *T. Spelta* var. *Duhamelianum* ♂.

The cytological characters of this hybrid proved exactly similar to those of the hybrids with *T. compactum* and *T. vulgare* (Figs. 144, 145).

Comparison of the cytology of the tetraploid and pentaploid hybrids of *A. cylindrica* with the wheats, reveals much that is common in the prophase, synizesis, and metaphase of the heterotype division, as well as in the second division and microspore formation.

The most striking difference, however, is observed in the number and morphological character of the bivalents in each class.

In the tetraploid hybrids mother cells are sometimes seen in which the diploid number of univalents is present in the heterotype division. Generally, however, there are a few bivalents, never more than 4 and usually from 1 to 3 of which most, if not all, appear to be of the end-to-end type.

In the pentaploid hybrids the number of bivalents is almost always 7. These are of the fused side-by-side type, presenting a characteristically different appearance, both in polar and profile views of the metaphase, from the bivalents of the end-to-end type in the tetraploid hybrids (cf. Figs. 106, 113 with Figs. 123, 124; also Figs. 100, 110, 117, 118 with Figs. 129, 130, 140, 141, 144, 145).

SERIES III. *AEGILOPS TRIUNCIALIS* L. ♀ × *Wheats* ♂.

The following hybrids in this series were studied:

(a) Tetraploid hybrids.

$$n = 14$$

$$n = 14$$

A. triuncialis L. ♀ × *T. dicoccoides* Körn.

var. *Kotschyianum* ♂.

var. *rubrivillosum* ♂.

A. triuncialis L. $\varnothing \times T. durum$ Desf.

var. *affine* δ . (Spain.)

$\times T. turgidum$.

var. *lusitanicum* δ . (Greece.)

(b) Pentaploid hybrids.

$n = 14$

$n = 21$

A. triuncialis L. $\varnothing \times T. vulgare$ Host.

var. *milturum* δ . (Austria.)

$\times T. Spelta$ L.

var. *album* δ . (Swiss.)

(a) Tetraploid hybrids.

A. triuncialis $\varnothing \times T. durum$ var. *affine* δ .

In this hybrid the dense synizetic knot opens out, and the diploid number (28) of chromosomes can be counted. Later, in the heterotype metaphase, from 1 to 6 end-to-end bivalents are found arranged on the central fibres of the spindle (Figs. 146-148). Movement of the chromosomes to the poles of the spindle takes place with some regularity, 14 univalents, single or divided, often reaching each pole; though in some cells one or two more travel to one pole than to the other. In this hybrid the homotypic split in the chromosome is generally delayed until late anaphase, and not infrequently one or two remain undivided (Fig. 149).

Univalents or their divided halves occasionally lag behind the rest and fail to become incorporated in the telophase nuclei of the dyad cell (Figs. 150, 151). They often remain isolated in the cytoplasm and are ultimately found in dwarf nuclei, as indicated in Fig. 152.

The second or homotype division usually appears normal, with clearly defined equatorial plates, but movement of the chromosomes from the plates is erratic, and although the mother cell is divided into typical 4-celled tetrads, the numbers of chromosomes in each microspore is not uniform.

A. triuncialis $\varnothing \times T. dicoccoides$.

var. *Kotschyannum* δ .

var. *rubrivillosum* δ .

$\times T. turgidum$.

var. *lusitanicum* δ .

In the early prophase of these hybrids separate chromosomes or pairs joined end to end by fine threads are seen.

The numbers and distribution of the chromosomes and other cytological features in these hybrids are similar to those of the preceding hybrid (Figs. 153-164). There is, however, apparently more regularity in the movement of 14 chromosomes to each pole of the spindle in both the heterotype and homotype divisions of some of the hybrids with *T. dicoccoides*, and tetrads with 14 chromosomes in each cell are frequent.

(b) Pentaploid hybrids.

A. triuncialis ♀ × *T. vulgare* var. *millurum* ♂.

In the early prophase double threads were seen (Fig. 165), doubtless formed by the coalescence of fine single threads as in some other hybrids in which the structure is more clearly apparent.

Condensation, shortening and thickening of the chromosomes rapidly follow, these appearing as separate structures. Later, the nuclear membrane disappears, and a dense synizetic knot is formed (Fig. 167). Very soon the knot opens out, and the diploid number (35) of short thick chromosomes can be counted, some of them being united end to end to form bivalents (Figs. 169, 170).

In the heterotype division from 1 to 5 end-to-end bivalents are found, 3 being a common number.

The chromosomes split homotypically in late anaphase of this division (Fig. 171).

At each pole of the cell there are often two groups, namely, a small group of 3 to 4 collected there at the time when the spindle is first formed, and a larger group consisting of chromosomes which move to the poles later. In the telophase the groups often remain distinct for a time (Fig. 172). There is great irregularity in the distribution of the chromosomes in the first division. Occasionally the dyad contains two nuclei, but in many instances there are a number of scattered nuclei, each embracing a different number of chromosomes (Fig. 173).

In the homotype division there is also much irregularity in the distribution of the chromosomes.

Some of the mother cells are divided into 4-celled tetrads, but the nuclei of each cell varies much in chromosome content. Micronuclei are common; and occasionally very small portions of cytoplasm are cut off the main bulk of the mother cell and develop into dwarf pollen grains (Figs. 174, 175).

A. triuncialis ♀ × *T. Spelta* var. *album* ♂.

The cytology of this hybrid closely resembles that of the preceding one. Bivalents are comparatively rare, but 3 or 4 of the end-to-end type are sometimes seen.

Irregular distribution to the poles, and lagging of chromosomes occur both in the heterotype and homotype divisions (Figs. 176–185).

SERIES IV. *AEGILOPS VENTRICOSA* ♀ × WHEATS ♂.

The following hybrids in this series were studied:

(a) Triploid hybrid.

$n = 14$

$n = 7$

A. ventricosa Tausch. ♀ × *T. monococcum* L. ♂.

(b) Tetraploid hybrids.

$n = 14$

$n = 14$

A. ventricosa Tausch. ♀ × *T. dicoccoides* Körn.

var. *Kotschyannum* ♂.

× *T. dicoccum*.

var. *farrum* ♂. (France.)

× *T. polonicum* L. ♂.

× *T. turgidum* L.

var. *lusitanicum* ♂. (Greece.)

(a) Triploid hybrid.

Aegilops ventricosa Tausch. ♀ × *Triticum monococcum* L.

Several grains were obtained from the hybridisation of *Aegilops ventricosa* and *Triticum monococcum*. Some of the F_1 plants grew well, but the culms ended in inflorescence axes at the notches of which either no spikelets were produced, or only rudimentary spikelets possessing small glumes within which no flowers were developed. Others of the F_1 plants gave inflorescences with spikelets containing flowers with full grown anthers. All the plants were sterile.

Meiosis in the pollen mother cells was investigated. In the earliest stage examined the nucleus contains chromosomes of different lengths, a densely staining nucleolus and a clearly defined nuclear membrane. Contraction of the nucleus and its contents follows, and a compact synizetic knot is produced (Figs. 186, 187).

The knot soon opens out, and short, thick chromosomes begin to take

their place on the spindle (Fig. 188). In some of the cells the diploid number of univalents is seen, while in others from 1 to 4 bivalents are found. Some of the bivalents consist of 2 chromosomes joined at both ends, the pair forming an oval ring, the majority, however, are formed of 2 univalents joined end to end.

In the metaphase of the heterotype division, the bivalents occupy the central part of the spindle, the separate univalents being scattered irregularly in the cell (Figs. 189-191). The halves of the bivalents travel to opposite poles where they join the univalents which are already there or which move there later, the number of chromosomes collecting at each pole varying from 9 to 12. Lagging chromosomes are somewhat rare.

Practically all the univalents are homotypically split in the anaphase of the heterotype division, the split appearing soonest in cells which contain no bivalents (Fig. 192). Separation of the monads or halves of the split chromosomes only occurs in lagging univalents (Fig. 193), in which cases the monads are distributed to opposite poles.

In the homotypic division the metaphase is comparatively regular, and in polar views both dyad (univalents) and monad chromosomes are frequently seen on the equatorial plate (Figs. 195, 196). Only the dyad chromosomes now divide their halves being distributed in the anaphase to the poles of the spindle along with the monads arising in the previous division. The separating chromosomes move irregularly, some of them lagging (Fig. 197) and failing to become included in the nuclei of the microspores.

Tetrads are 4-celled, but owing to the irregular distribution of the chromosomes both in the first and second divisions of the mother cell, the number in each microspore varies from 4 or 5 to 16 or 18, the majority receiving from 9 to 12.

(b) Tetraploid hybrids.

A. ventricosa ♀ × *T. dicoccum* var. *farrum* ♂.

In the early prophase the segmented spireme undergoes condensation which culminates in the dense synizetic knot (Figs. 198, 199). The latter opens out rapidly, and soon the diploid number (28) of short, thick chromosomes separate from each other, and become irregularly distributed in the cell. A clearly defined metaphase with all the chromosomes on an equatorial plate is not seen. Bivalents are exceptional, and more than one in a cell is rarely observed. When present they are of the end-to-end type.

In the heterotype division the formation of a bipolar spindle with the subsequent production of a dyad from the mother cell is comparatively uncommon. Tripolar spindles are, however, numerous (Figs. 207–209), and the division of the mother cell into a triad at this stage of meiosis is very frequent. Occasionally quadripolar spindles are found (Fig. 214), the mother cell being then divided into a tetrad at the first division.

The distribution of the univalents in the heterotype division is irregular. In cells with bipolar spindles the number which travel to opposite poles is occasionally 14, or exactly half the diploid number, but variations between 7 at one pole and 21 at the other are more usual. In cells with tripolar spindles there is also considerable irregularity (Figs. 207, 208), although 7 univalents at each of two poles with 14 at the third is sometimes seen (Fig. 209).

In the anaphase of this division the chromosomes undergo the homotypic split often with clear separation of the halves (Figs. 201, 202).

In the homotypic division regular metaphase equatorial plates are frequent, but lagging chromosomes in the anaphase are common (Fig. 202).

The dyad resulting from the heterotype division is followed by the production of an ordinary 4-celled microspore tetrad (Fig. 206).

The homotypic division which follows the formation of a tripolar spindle and the production of a triad in the heterotype division brings about the division of the mother cell into 6 microspores, all approximately equal in size (Figs. 211–213).

Similarly the mother cell is finally divided into 8 microspores when distribution of the chromosomes on a quadripolar spindle occurs in the heterotype division (Figs. 214, 215).

A. ventricosa ♀ × *T. dicoccoides*.

var. *Kotschyannum* L.

× *T. turgidum*.

var. *lusitanicum* ♂.

× *T. polonicum* ♂.

The cytology of these tetraploid hybrids is similar to that of the hybrid described above (Figs. 216–238).

Both bipolar and tripolar spindles arise at the first division of the mother cell, and bivalents are exceptionally rare or absent.

DISCUSSION.

Heterotype prophase.

In the earliest prophases examined the nuclear cavity contains a spireme of exceedingly fine leptotene threads. The latter are discontinuous, with no sign of a split, and the separate threads are distributed at random throughout the nucleus without anything more than rare accidental parallelism among them.

Slightly later the nucleus contains fine double, discontinuous filaments. On account of the tenuity of the threads and filaments, and the coiling which takes place, their number cannot be counted. The number of double filaments, however, is clearly smaller than that of the unsplit threads preceding them, and supports the conclusion that the double filaments are formed by the close parallel approximation of the finer single threads of the spireme (Figs. 25-27, 135-137, 165).

After the formation of the double filaments, contraction and condensation of the nuclear contents occur, the filaments becoming shorter and thicker, and the line of union of the fine components is obliterated. In the nucleus at this stage are seen short thick chromosomes, and where their number can be counted there are either, (1) the diploid number of separate chromosomes (univalents) all approximately of similar length; (2) a number of short chromosomes as in (1), together with a variable number double the length of the rest, which I interpret as bivalents formed by the union of two univalents end to end (Figs. 106, 113, 138); (3) a number of short chromosomes as in (1), with seven or sometimes fewer bivalents which appear to be formed by side by side pairing of univalents (Figs. 124, 128, 142). In addition to the chromosomes the nucleus contains a deeply staining nucleolus, all within a clearly defined nuclear membrane. Considered separately, the nuclear figures at this period are readily mistaken for examples of ordinary diakinesis following synizesis, especially where side-by-side bivalents are seen as in Fig. 128. That this is not the correct interpretation is clear from careful observations of the sequence of changes in adjacent nuclei in the same anther loculus. The number of chromosomes is usually diploid or diploid minus a small number of bivalents, not haploid, and the condition described precedes synizesis. Contraction and condensation of the chromosomes follows, and a compact synizetic knot is formed, which appears imbedded in dense cytoplasm from which all trace of nuclear membrane has disappeared. The outlines of the individual chromosomes

are completely lost except where portions or loops are sometimes seen projecting from the knot. The prophase of the heterotype division may be taken to end at this stage.

Heterotype metaphase.

The synizetic knot in some respects is reminiscent of some forms of "second contraction" such as are observed in *Oenothera* and other plants, for after a short rest it opens out, and emerging from it in a large number of instances are well-defined univalent chromosomes, diploid in number. These become distributed irregularly over the spindle which forms at the same time. In certain hybrids, in addition to univalents, there are variable numbers of bivalents which arrange themselves in regular order on the central spindle fibres. Metaphases of the former type conform to the semi-heterotype division described by Rosenberg⁽¹⁰⁾ in *Hieracium laevigatum*, while the latter agree with Rosenberg's *Hieracium boreale* type. Since both are not uncommonly seen in heterotype divisions of the same hybrid, there appears to be no ground for regarding them as distinct types of division.

A peculiar feature of the metaphase is the rarity of any clearly marked equatorial plate in which all the chromosomes are collected.

Where only univalents are present these are generally scattered at random throughout the nucleus from one pole to the other, a few being very often found off the spindle in the cytoplasm. When bivalents are found, these take up a definite position in the equatorial zone on the central fibres of the spindle, where they are frequently surrounded by an outer ring of univalents. An exception to the generalisation just made was met with in the cross *A. ovata* \times *T. dicoccum* var. *persicum* (Black Persian wheat) in which good metaphase plates, with all the chromosomes closely arranged in the equatorial zone, were frequent. I observed similar examples occasionally in *A. cylindrica* \times wheat hybrids, and they have also been recorded by Sax⁽¹¹⁾ and Kagawa⁽⁷⁾, but they are rare.

A further peculiarity of the heterotype division is the occurrence of tripolar and quadripolar spindles in some of the crosses.

Bipolar spindles are normal in hybrids of *A. cylindrica* \times wheats and of *A. triuncialis* \times wheats. In the hybrids of *A. ovata* \times wheats, in addition to the normal bipolar spindles, tripolar and occasionally quadripolar spindles occur. These are especially numerous in hybrids of *A. ventricosa* \times *T. dicoccoides*, *T. dicoccum*, and *T. turgidum*, but none were observed in the cross *A. ventricosa* \times *T. monococcum*. Reference is made later to these and other peculiar cytological features, and to their

bearing on the problem of the relationship of the wheats to species of *Aegilops*.

The bivalents in *Aegilops* \times wheat hybrids are of two distinct forms, namely (1) telosyndetic bivalents composed of two univalents joined end to end by a very fine, short thread (Figs. 6, 7, 116, 158), and (2) parasyndetic bivalents consisting of two univalents arranged and subsequently fused together in a side by side position. The latter in polar view appear as single chromosomes double the thickness of the associated univalents (Figs. 128, 142), and in profile at early anaphase take the form of oval or elliptical rings (Figs. 130, 290, 291).

The differences indicated in the character of the bivalents are most clearly seen in the heterotype metaphase, but, as previously noted, they are already recognisable in the prophase before the formation of the synizetic knot. A study of the nuclei at this earlier period suggests that both telosyndetic and parasyndetic bivalents arise through the union of two univalents end to end in a more or less straight line. In the parasyndetic type, however, the two components become later bent round at their point of contact until they lie parallel to each other, the free ends sometimes uniting so that the whole forms an oval ring; while the components of the telosyndetic pair remain longitudinally aligned, in which position they are ultimately arranged on the spindle with their free ends directed towards the poles (Figs. 8, 118, 148, 259).

In *Aegilops* \times wheat hybrids, unequivocal parasyndetic bivalents have only been found in the crosses, *A. cylindrica* \times Bread, Club and Dinkel wheats (*T. vulgare*, *T. compactum* and *T. Spelta*) respectively; in the hybrids *A. ovata* \times wheats and *A. triuncialis* \times wheats, the bivalents are all of the telosyndetic type, as they are also in *A. ventricosa* \times wheats, except *A. ventricosa* \times *T. monococcum* where a small number of both kinds are seen.

Heterotype anaphase.

In many cells of all the hybrids studied, the diploid number of univalents is seen at the heterotype metaphase. These are irregularly distributed in the cell, it being an exceptionally rare occurrence to find all the chromosomes collected on a clearly circumscribed metaphase plate. In a number of cells, however, of most of the hybrids, from 1 to 7 bivalents are seen: the maximum number found in the different hybrids varies, and is dependent on the affinity of the two parents crossed, the particular number found in any cell when less than the maximum for the

hybrid, being apparently determined by accidental cytological conditions within it.

In cells in which only univalents are present, the chromosomes are distributed irregularly from the metaphase position to the two poles; details are given previously under the several hybrids.

Where bivalents are present, the components separate in normal manner, one going to each pole.

Lagging of chromosomes is a common feature of the anaphase, the general arrangement in three groups in the cell resembling that seen in the classical *Drosera* hybrids; this feature, however, occurs not only in triploid and pentaploid hybrids whose parents have a different haploid number, but also in tetraploid hybrids in which both parents supply an equal number of chromosomes, namely fourteen. As previously noted in such cases where non-pairing occurs, and the diploid number of non-homologous univalents is seen in the metaphase, I am of the opinion that units of the chromosome complement of the mother parent (*Aegilops*) move first, and more or less regularly, to the poles, leaving the irregularly scattered and lagging chromosomes of the wheat parent to follow later, or be left, as they sometimes are, isolated in the "foreign" cytoplasm off the spindle.

A characteristic cytological feature in the anaphase of the heterotype division is the longitudinal homotypic splitting of all the chromosomes, except a small number found near the poles or in the cytoplasm off the spindle, which may remain undivided until the second division, or throughout the whole meiotic phase.

This phenomenon is a constant feature of all the hybrids examined. The splitting takes place soonest in cells in which only univalents are present, the chromosomes being divided in the earliest anaphase. In cells containing bivalents the splitting is delayed until the "reduction" or separation of the components of the bivalents is completed, the delay being longest in cells possessing the greatest number of bivalents. Similar longitudinal splitting of univalents in the first division has been recorded by Kagawa⁽⁷⁾ in the hybrid *A. cylindrica* \times *T. vulgare*; but, on the other hand, Sax⁽¹¹⁾ observed no such splitting in a similar hybrid, possibly owing to the delay which is general in cells containing seven parasynthetic bivalents.

In nearly all of the very large number of cases investigated in these researches, the division of the univalent chromosomes is always clearly seen in late anaphase and early telophase of the heterotype division. Many of the halves of the longitudinally divided chromosomes remain

together, both travelling to the same pole, but the halves of some of them separate completely from each other and move to opposite poles, the numbers behaving in this manner varying in different hybrids. (See the figures and details previously given.)

The first division in the ordinary meiotic phase appears to be concerned only with the separation of the components of bivalents, the second division being a simple mitosis or equational division of univalents which were separated in the previous division. In these *Aegilops* \times wheat hybrids, however, in which there are few or no bivalents to "reduce" and only univalents are present in the metaphase of the first division, the equational division begins at this stage of meiosis instead of being reserved for the second division.

The occurrence of the diploid number of univalents in the heterotype metaphases in these hybrids, and their longitudinal division at this stage, appears to have a direct bearing on the elucidation of the nature of the leptotene threads and their parallel conjugation observed in the early prophases. Parallel pairing and complete coalescence or fusion of the leptotene threads undoubtedly occurs in the early prophases of these hybrids, and although the number of threads present in the dividing cells at the time of their conjugation cannot be determined with certainty, in the later stages of the prophases when they can be counted, there always appears the diploid, never the haploid, number demanded by the hypothesis that the double structures are bivalents produced by the conjugation of threads representing whole chromosomes and not univalents composed of halves of chromosomes divided in the preceding telophases.

In many of the cells of the post-synizetic heterotype metaphase, the diploid number of univalents is again seen, and these split longitudinally and the halves often separate in the anaphase. It would appear that such division and separation can only represent the separation of the individual leptotene threads which united in the early prophase, a conclusion supporting the view of Farmer, Digby⁽³⁾ and others regarding the nature of the pairing of threads in the early prophases in the plants which they investigated.

Interkinesis.

In the telophase of the heterotype division the chromosomes in some cases pass over into a vesicular resting nucleus of normal form; in many hybrids, however, two or more groups are seen in the telophase, each group consisting of a variable number of chromosomes which have

travelled at different rates to the poles. Sometimes the groups remain separate and form resting nuclei of variable number and size, or they coalesce more or less into nuclei of irregular shape (Figs. 58, 59, 103, 172, 173, 220).

In all cases of interkinesis where a bipolar spindle arises in the pollen mother cell, the latter is divided into two by a cell wall; whereas when tripolar or quadripolar spindles are formed the cell is divided at this stage into three or four parts.

The homotypic division.

After a short interkinetic pause, the homotypic division commences. In this division in all the hybrids, no matter how irregular the previous division has been, the chromosomes arrange themselves at the metaphase on a well ordered equatorial plate. Movement away from this position, however, is in all cases irregular, some of the chromosomes travelling off the plate towards opposite poles before the rest which remain behind as a lagging group in the equatorial zone. This irregularity is doubtless connected with the presence in the nuclei of univalents which passed undivided or only split to the poles in the previous division, and monads or halves of univalents which divided and separated at the same time to opposite poles. The "laggers" move so slowly and irregularly that some of them fail to become included in the tetrad nuclei, a single one remaining isolated in the cytoplasm of the cell, while two or three when thus left often join to form micronuclei in the pollen grain.

Four-celled tetrads are frequent in most of the hybrids, but the chromosome complement of each cell varies considerably, rarely consisting of an equal number. In addition to 4-celled tetrads, 5- to 6-celled tetrads are common in the hybrids of *A. ovata* \times all wheats, these originating from mother cells in which there are tripolar spindles in the heterotype division; they are also found in hybrids of *A. cylindrica* \times Bread wheat series, in which tripolar spindles are likewise present.

In the hybrids of *A. ventricosa* \times Emmer series, tetrads, hexads and octads are found, the hexads in the cells in which tripolar spindles occur, the tetrads and octads in cells having bipolar and quadripolar spindles in the heterotype metaphase.

In the hybrids of *A. triuncialis* \times Emmer and Bread wheat series, *A. cylindrica* \times Emmer series, and *A. ventricosa* \times *T. monococcum*, only 4-celled tetrads have been noticed, and it is in these hybrids that tripolar spindles are very rare or entirely absent in the heterotype division.

As previously observed, each of the four cells of a tetrad rarely receives

an equal number of chromosomes; very frequently two nuclei are present in each cell, one of them a micronucleus originating from 2 or 3 chromosomes which remain off the spindle at the first division of the mother cell.

In some hybrids the 2 or 3 aberrant chromosomes induce a separation of a small portion of the cytoplasm from the bulk present in the cell, with the ultimate formation of a very small microspore. Such separation or splitting off of bits of cytoplasm is common in the hybrids of *A. ovata* × Emmer and Bread wheat series (Figs. 72, 73), and in those of *A. triuncialis* crossed with the same wheats (Fig. 175). Bits, however, are rarely cut off at the final division of the mother-cell in the hybrids of *A. cylindrica* × wheats, and *A. ventricosa* × Emmer series, and were not observed in the cross *A. triuncialis* × *T. Spelta*; possibly the few chromosomes which behave in this independent manner belong to the pollen parent as suggested previously.

The relationship of the wheats and species of Aegilops.

The races of wheat fall into three series, namely (1) the diploid Einkorn series with *T. monococcum* as the type, doubtless derived from the wild *T. aegilopoides* which it very closely resembles; (2) the tetraploid Emmer series consisting of *T. dicoccum*, *T. orientale*, *T. durum*, *T. polonicum*, *T. turgidum* and *T. pyramidale*, apparently springing from the wild prototype *T. dicoccoides*; (3) the hexaploid Bread wheat series with Bread wheat (*T. vulgare*) as the type and including *T. compactum*, *T. sphaerococcum* and *T. Spelta*, of which no wild ancestor is known.

A study of the morphology of the different races led me to the conclusion, expressed in my monograph "The Wheat Plant," that the wheats of the Emmer series are very closely related to each other. It appears also probable that the wild tetraploid prototype of Emmer, *T. dicoccoides*, may be phylogenetically connected with the wild diploid Einkorn prototype, *T. aegilopoides*. In the peculiar pubescence of the leaves and nodes, and in the form of the empty glumes the recently described *T. Timofeevi* of Zhukovsky (*T. dicoccoides* var. *Timofeevi*) and the Asiatic *T. aegilopoides* var. *Thaoudar* are exactly similar, and close morphological resemblance is observed between other varieties of these two wild species of *Triticum*, the differences chiefly relating to size and not to qualitative characters. I feel confident that *T. dicoccoides* is an autopolyploid arising from the doubling of the diploid species, a process which, while intensifying the features possessed by the mutating species, would not be likely to lead to the production of a plant with totally new characters.

In regard to the origin of the Bread wheats (*T. vulgare*) for which no prototype has been discovered, evidence was given in my monograph (p. 342) in support of the view that these are a vast collection of hybrids and their segregates, derived from the crossing of some of the Emmer series with *Aegilops ovata* and *A. cylindrica*, and continued study of the wheats and their hybrids with species of *Aegilops* emphasises this view. Prolonged investigations will be necessary before the particular influence of the assumed parental species can be clearly recognised in individual wheats of the race, nevertheless, the characteristic form of the grain, its starchiness, the round-backed empty glumes and special toughness of the rachis, the hollow, thin-walled straw and leaf structure of the Bread wheats are found in *A. ovata*; the winter habit, lateness of ripening, susceptibility to attacks of *Puccinia glumarum* and beardlessness of many forms of Bread wheats, as well as the characteristic fracture of the rachis of *T. Spelta*, point to *A. cylindrica*, while the strongly bearded feature, keeled glumes, partial resistance to rust, as well as earliness and the upright, spring habit of some forms may be attributed to the *Triticum* parent.

As previously observed, the scheme of investigations undertaken had for one of its objects the production and examination of a series of hybrids between the four tetraploid species (*Aegilops ovata*, *A. cylindrica*, *A. triuncialis* and *A. ventricosa*) and representatives of the diploid, tetraploid and hexaploid races of wheats. It was hoped that the study of the cytology of the hybrids would throw light on the relationships between the plants concerned, and at the same time add to the knowledge of the cytology of hybrids in general.

Unfortunately the chromosomes of wheats and of the species of *Aegilops* used, as seen in meiosis, are too much alike to allow of their being accurately recognised in dividing cells; and present knowledge of the cell, extensive as it is, is not sufficient to admit of the deduction of plant relationships from an examination of the cytological details of their hybrids; nevertheless, some of the results of these investigations, have, I think, a bearing on the problems indicated.

The diploid chromosome number of the species of *Aegilops* used in the researches is 28, that of the wheats being 14, 28 and 42 for the Einkorn, Emmer and Bread wheats respectively.

Accepting the connection between morphological characters, genes and chromosomes, the tetraploid Emmer series may well be autopolyploids originating from the Einkorn wheats by mutation involving a doubling of the diploid chromosomes of the latter, for the primitive

forms of both series are very similar in qualitative morphological characters. On the other hand, the hypothesis that the hexaploid Bread wheats with 42 chromosomes may have been derived from a process of doubling and crossing of Einkorn with 14 and representatives of the Emmer wheats with 28 chromosomes can be dismissed; for it is hardly conceivable that repetition or addition of the chromosomes of these two series could result in the production of the totally new characters found in the Bread wheats: for these, the introduction of a qualitatively different set of chromosomes is needed.

Examination of the cytological details exhibited by the *Aegilops* × wheat hybrids reveals some peculiarities which I regard as significant of the genetical relationships of the plants. Among the most striking features discovered are (1) the occurrence of two distinct types of bivalents; (2) variation in the total and relative numbers of bivalents of the two types in the several hybrids; (3) the formation of tripolar and quadripolar, in addition to the normal bipolar spindles. The distribution of these features in the hybrids is indicated below.

Spindles		Hybrid	No. of bivalents	Kind of bivalent
Bipolar and tripolar	<i>A. ovata</i> ♀	× <i>T. monococcum</i> ♂	1-5	Telo.
		× <i>T. dicoccoides</i> ♂	1-3	"
			(usually)	
		× <i>T. dicoccum</i> ♂	1	"
		× <i>T. dicoccum</i> var. <i>persicum</i> ♂	0-1	"
		× <i>T. polonicum</i> ♂	1-2 (2 rare)	"
		× <i>T. turgidum</i> ♂	1-2	"
		× <i>T. durum</i> ♂	1-2	"
		× <i>T. vulgare</i> ♂	2-3	"
		× <i>T. compactum</i> ♂	2-3	"
		× <i>T. Spelta</i> ♂	1-3	"
Bipolar, tripolar, rare	<i>A. cylindrica</i> ♀	× <i>T. dicoccoides</i> ♂	1-4	Telo.
			(usually 2)	
		× <i>T. dicoccum</i> ♂	1-4	"
		× <i>T. polonicum</i> ♂	1-4	"
		× <i>T. turgidum</i> ♂	1-4	"
		× <i>T. vulgare</i> ♂	7	Para.
		× <i>T. compactum</i> ♂	7	"
Bipolar, tripolar, none, or very rare	<i>A. triuncialis</i> ♀	× <i>T. dicoccoides</i> ♂	1-3	Telo.
		× <i>T. durum</i> ♂	1-6	"
		× <i>T. turgidum</i> ♂	1-3	"
		× <i>T. vulgare</i> ♂	1-5	"
		× <i>T. Spelta</i> ♂	0-3 (rare)	"
Bipolar only. Tripolar very numerous, a few quadripolar	<i>A. ventricosa</i> ♀	× <i>T. monococcum</i> ♂	1-4	Telo. and para.
		× <i>T. dicoccoides</i> ♂	0-2	Telo.
		× <i>T. dicoccum</i> ♂	0-2 (very rare)	"
		× <i>T. turgidum</i> ♂	0-2	"
		× <i>T. polonicum</i> ♂	0-2	"

The complete interpretation of the peculiarities tabulated above cannot yet be attempted; much more investigation is needed before this is possible. Some of the results, however, are suggestive and may be considered now.

Discussing first the differences in the kind and number of bivalents and their significance in the several hybrids, it may be assumed that absence of pairing of the univalents during meiosis is evidence that the chromosomes of the two parents are either not at all or only remotely related. On the other hand, pairing, whether para- or telosyndetic, is indicative of some kind of homology in the uniting chromosomes.

From a consideration of meiosis as seen in the majority of flowering plants and the special study of the cytology of these hybrids, I conclude that parasyndetic or side by side bivalents only result from the parallel conjugation and fusion of exactly homologous chromosomes, and are the normal type in all self-fertilised and cross-fertilised plants of which the uniting parents belong to the same species.

That such a mode of pairing is indicative of the closest homology, and distinct from end to end pairing, is clear from a comparison of the cytological differences found in the sterile and the fertile hybrids of *Aegilops ovata* \times *T. dicoccoides* respectively, and in the sterile and the fertile hybrids of *A. ovata* \times *T. turgidum*.

In the heterotype division of the sterile tetraploid hybrids which I obtained between *A. ovata* ($n = 14$) and two varieties of *T. dicoccoides* ($n = 14$) only 1 to 3 bivalents are found, and these when present are of the end-to-end type, the remaining 20 to 26 univalents do not pair. In the fertile octoploid hybrid produced by Tschermak (13) between the same species, Bleier (1, 13) found that doubling of the chromosomes had taken place, the diploid number being 56 or twice the diploid number of the parents *A. ovata* and *T. dicoccoides*.

The haploid number of chromosomes of each gamete of the hybrid is 28, consisting of a set of 14 derived from *A. ovata*, and a similar set of 14 from *T. dicoccoides*. At meiosis 28 bivalents should therefore be formed since for each chromosome there is an exactly homologous mate with which to pair: moreover, on the hypothesis suggested, all should be of the parasyndetic type.

I have investigated the meiotic phase in Tschermak's fertile hybrid and find this is the case, the metaphase plate showing 28 fused bivalents, most, if not all, of which in the prophase appear as oval rings (Fig. 250).

Analogous cytological features are found in the sterile tetraploid

hybrid, *A. ovata* \times *T. turgidum* var. *mirabile*, and the corresponding fertile hybrid of the same species (pp. 208, 236 and Figs. 46, 47, 240).

Similarly in the F_1 of the hybrid *A. ovata* \times *T. dicoccum* there are few or no homologous chromosomes, and no pairing occurs, 28 univalents being seen in the prophase and metaphase of the heterotype division. Sax(12), however, observed 14 parasynthetic fused bivalents and 14 univalents in the heterotype division of the back-cross hybrid *A. ovata* \times *T. dicoccum* (F_1) pollinated with *T. dicoccum*. Presumably the ovum of *A. ovata* \times *T. dicoccum* (F_1) was unreduced and possessed 28 chromosomes, 14 from the Emmer and 14 from the *Aegilops* parent. On crossing with Emmer pollen the 14 chromosomes of the latter find an exactly homologous set with which to conjugate, and the pairing is therefore parasynthetic.

In the light of what has been stated above, the hybrids produced by crossing *A. cylindrica* with the Bread wheat series are of special interest. The presence of a constant number (7) of parasynthetic bivalents in these hybrids, and the complete absence of this type of bivalent in the hybrids of *A. cylindrica* crossed with wheats of the Emmer series, I take to be indicative of a real difference between the Emmer and the Bread wheats, and also very strong evidence that seven of the univalents of *A. cylindrica* have their exact homologues in the chromosome complex of the hexaploid Bread wheats.

Moreover, both the number and type of bivalent found in the *A. cylindrica* \times Bread wheat hybrids are cogent testimony in support of the conclusion that the Bread wheats have arisen by hybridisation, and that one of the parents is *A. cylindrica*, a deduction arrived at independently ten years ago from a study of the morphology of these wheats.

The significance of telosynthetic pairing seen in the rest of the *Aegilops* \times wheat hybrids, and the complete absence of pairing of the chromosomes observed in some hybrids, although not yet clear in certain points, may be considered in the light of the knowledge obtained in these and other cytological researches.

In hybrids in which only univalents are found in the heterotype division, i.e. where there is complete absence of pairing, it may be confidently assumed that the parents belong to distinctly different species.

This view is corroborated by the investigations of the hybrids *Crepis setosa* \times *C. capillaris* (Collins and Mann(2)), *Digitalis purpurea* \times *D. lutea* (Haase-Bessell(5)) and *Raphanus sativus* \times *Brassica oleracea* (Karpechenko(8)). No doubt about the specific difference of the parents

is entertained by taxonomists, and in the hybrids there is no pairing of chromosomes, only univalents, diploid in number, being observed in diakinesis and the heterotype metaphase.

The meaning of telosyndetic pairing seen in all the *Aegilops* × wheat hybrids except *A. cylindrica* × Bread wheats, is not so clear.

As previously observed, both telosyndetic and parasyndetic bivalents appear to arise in the same way, namely, by the end to end union of single chromosomes; but in the latter type the components, during the prophase, bend round so that they lie parallel to each other, the free ends uniting to form an oval ring, the enclosed space between the chromosomes being frequently obliterated later by complete lateral fusion.

In the telosyndetic bivalents the components remain aligned end to end throughout the prophase, unfolding from synizesis and taking their place so aligned on the spindle fibres at metaphase. I conclude that telosyndetic pairing denotes a more remote relationship or homology, between the components of the bivalents uniting in this manner, than that between the chromosomes which conjugate parasyndetically.

Taking a wide survey of the results exhibited by these hybrids, and also of the cytological features of the *Aegilops* species crosses described later, the suggestion arises that telosyndetic pairing is indicative of a relationship between the uniting chromosomes similar to that recognised by the taxonomist between sub-species and different yet closely related species.

Possibly the end to end arrangement of the chromosomes and rarity of parasyndetic bivalents in meiosis of *Oenothera*, is in some way analogous to the telosyndetic pairing referred to here, and that some of the so-called species of *Oenothera* are really hybrids between nearly related species.

So far as relationships are concerned in the species of *Aegilops* and the wheats which have been examined in these researches, there are three classes of chromosomes, namely:

- (1) Exactly homologous chromosomes, which pair and become fused along their whole length;
- (2) Chromosomes uniting end to end only, denoting sub-specific or closely allied specific relationship; and
- (3) Non-pairing chromosomes belonging to truly different species.

From a study of the tables on p. 231 it is seen that the chromosome complement of the several species of *Aegilops* and wheats is composed of a variable number of chromosomes of the three classes. In none of the hybrids are all of the chromosomes paired in either way; some of them

exhibited pairing of the para- or telosyndetic type, but many chromosomes behave as belonging to distinctly different species, the number of each class being readily deduced from the results given in the tables.

Five out of the seven chromosomes of *T. monococcum* exhibit telosyndetic relationship with 5 of the 14 of *A. ovata*. Three of the 14 in *T. dicoccoides* find similar mates in the *Aegilops* parent, while only one or two present in the cultivated wheats of the Emmer series show the same affinity with a similar number in *A. ovata*.

In the majority of the Wheats of the Bread wheat series usually 2 or 3 out of the 21 are related to chromosomes of *A. ovata*, but in *T. sphaerococcum* 4 often pair with 4 from the *Aegilops* parent.

From the results of the hybrids of *A. cylindrica* with the wheats, it is inferred that of the 14 chromosomes of the *Aegilops*, from 1 to 4 have telosyndetic mates in *T. dicoccoides*, but only 2 find similar mates in the cultivated Emmer series.

In contrast is the discovery that 7 of the 14 chromosomes of *A. cylindrica* find exact homologues among the 21 chromosomes of the Bread wheats, the bivalents being of the parasyndetic type: reference has already been made to the significance of this result in its bearing on the origin of the Bread wheats.

The number of bivalents in the hybrids of *A. triuncialis* with wheats of the Emmer series, is usually 3 to 6, or somewhat higher than the number found in the hybrids of *A. ovata* or *A. cylindrica* with the same wheats; 5 telosyndetic mates are frequently found in *T. vulgare* but bivalents are rare in the cross *A. triuncialis* \times *T. Spelta*. These differences may be related to the probable hybrid origin of *A. triuncialis* discussed later.

The chromosomes of *A. ventricosa* appear to be specifically distinct from those of the cultivated Emmer series of wheats, for pairing is exceptionally rare or absent in their hybrids; occasionally one or two telosyndetic bivalents are observed in the hybrids with the wild *T. dicoccoides*, but they are rare. On the other hand, from 1 to 4 bivalents, some telo- other parasyndetic, are seen in the hybrid *A. ventricosa* \times *T. monococcum*.

The third peculiar cytological feature (p. 222) encountered in these hybrids, more particularly in some than others, is the frequent occurrence of tripolar and occasional quadripolar spindles in the heterotype divisions. This somewhat unusual feature has often been recorded in investigations of the cytology of hybrids, but its significance does not appear to have been examined or discussed. In the majority of the

hybrids of *Aegilops* sp. × wheats the spindle is bipolar, but in the crosses of *A. ventricosa* with all the wheats of the Emmer series, tripolar spindles are very numerous. The diploid number (28) of univalents is generally present, and these arrange themselves in three groups when the spindle is tripolar, the numbers moving to each pole varying usually from 8 to 11. In a few instances (Fig. 209) however, 7 chromosomes travel to each of two poles, and 14 to the remaining third: this is highly suggestive that there is a clear separation of the two parental groups of 14 chromosomes from each other, one group of 14 collecting at one pole, while the remaining group of 14 from the second parent distribute themselves evenly to the other two poles, 7 going to each. Unfortunately the chromosomes of the parents are too much alike to allow of their certain recognition, but it appears likely that the two groups of 7 univalents which move in regular order away from each other to two of the poles are *A. ventricosa* chromosomes which find themselves at home in the nuclear surroundings of their mother cell, the 14 wheat chromosomes of the pollen parent being aliens in the nucleus and cytoplasm, and less likely to be controlled than those normally present.

Tripolar spindles are occasionally found in all the hybrids of *A. ovata* × wheats; on the other hand they are rare in the hybrids of *A. cylindrica* × wheats, and *A. triuncialis* × wheats and were not seen in the hybrid *A. ventricosa* × *T. monococcum*.

The significance of these differences is obscure; possibly, it may ultimately be shown that the constant occurrence of tripolar or quadri-polar spindles is correlated with the presence in the cell of at least two sets of non-homologous chromosomes in more or less equal numbers.

That homology exists among the chromosomes of the wheats and of the species of *Aegilops* used in these researches, is, I think, clear. The results, however, in regard to the amount and kind of pairing of the chromosomes in the hybrids, are at present too complex to be reduced to formulae; there is, however, little doubt that investigations of the morphology of the chromosomes and carefully planned studies of the cytology of hybrids will ultimately lead to analysis of the relationships existing between species which can be crossed.

A FERTILE *AEGILOPS*-*TRITICUM* HYBRID.

Among the many crosses made in 1926 were the following:

- (1) *A. ovata* × *T. turgidum* var. *mirabile*.
- (2) *A. ovata* × *T. turgidum* var. *iodurum*. (Poulard d'Australie.)

The F_1 of the former at harvest of 1927 proved quite sterile, and the

cytology similar to that of the hybrid *A. ovata* \times *T. dicoccum* and other tetraploid hybrids of *A. ovata* crossed with wheats of the Emmer series.

The chromosome number of the F_1 plant was 28, which was determined by an examination of somatic cells as well as the pollen mother cells.

The distribution of the chromosomes, chiefly univalents, was irregular, from 12 to 18 univalents or their divided equivalents moving to the poles of the heterotype spindle. The homotype division led to the production of tetrads and hexads, the individual cells of which possessed from 4 to 8 or 9 chromosomes.

Examination of the mother cells of the second hybrid with Belling's aceto-carmines also showed 28 univalents present. At harvest the plant ripened five or six ears on long good straws, and three smaller ears on short straws less than half the height of the rest. The ears on the early well-developed straws were completely sterile, but four grains were produced on the short weak straws, two from one ear and one each from the others.

The four grains from this F_1 plant were sown in 1928 at the end of January in pots in the greenhouse, and two of them grew to maturity in the same season. One of the plants of this F_2 generation grew strongly and ripened well-developed, typical, hybrid ears, exactly like those of the F_1 plant; these were quite sterile. The second F_2 plant was attacked by wireworms (*Agriotes lineatus*) when it had sent up three or four leaves, three of the leaves being eaten off below ground and destroyed. After the wireworms were removed the plant began a fresh development, but took a long time to recover. Ultimately it produced several good ears exactly like those of its sister plant, but some of the ears bore several well-developed grains.

The pollen mother cells of both the sterile and fertile F_2 plants were found to possess 27 to 28 bivalents instead of 28 univalents observed in the mother cells of the early ears of the F_1 plant which were examined, and of the hybrids of *A. ovata* crossed with *T. turgidum* var. *mirabile* and other wheats of the Emmer series.

In the heterotype metaphase both bivalents and univalents are generally seen (Figs. 239-241). As in all the *Aegilops* \times wheat hybrids investigated, the univalents, with few exceptions, are all divided longitudinally in the anaphase of the first division, and 112 monads appear, scattered irregularly throughout the cell, though small, more densely crowded groups are usually found at the poles, these arising from the division of univalents which have reached the poles before being joined by the lagging chromosomes (Fig. 242).

From observations of the telophases of the heterotype division there appears to be a fairly equal distribution of the chromosomes to the two poles, aberrant individuals left in the cytoplasm being of rare occurrence at this stage of meiosis (Fig. 243).

In the homotype division 56 monads can often be counted on a clearly circumscribed metaphase plate (Fig. 244); movement, however, from this position is irregular, and in late anaphases lagging groups consisting usually of 7 or 14 chromosomes are frequent (Figs. 245, 246).

Single chromosomes or collections of two or three are often found off the spindle in the final division, and when failing to become incorporated in the main nucleus form micronuclei in the cells of the tetrad (Figs. 247, 248).

The cytology of this fertile hybrid is exactly similar to that observed by Bleier (1, 13) in Tschermak's fertile (*Aegilotriticum*) hybrids *A. ovata* × *T. dicoccoides* and *A. ovata* × *T. durum* Arraseita var. *Hildebrandtii* (= a purple-grained Abyssinian variety of *T. dicoccum*).

I have grown both these at Reading from grains kindly supplied by Prof. Tschermak and have confirmed Bleier's results. On the metaphase plate of the heterotype division 28 typical parasynthetic bivalents are frequently seen (Fig. 249); in the late heterotype prophase (? diakinesis) the two components of the bivalents are united at both ends to form oval rings or are joined at one end and bent round in the shape of a horseshoe, in which condition they often pass on to the spindle fibres (Figs. 250, 251).

Much interest attaches to the occurrence of the double number of chromosomes in the somatic tissues and gametes of these fertile *Aegilops* × wheat hybrids, and while some points regarding the mode of origin and time of the change remain obscure, others are clear in respect of the hybrid *A. ovata* × *T. turgidum* var. *iodurum*.

The doubling was first observed in the F_2 generation, 56 chromosomes being found in the somatic and pollen mother cells; the number in those mother cells examined of the F_1 plant had 28 as expected, this number being also found in all the hybrids of *A. ovata* ($n = 14$) × other wheats of the Emmer series ($n = 14$). It would appear that the doubling of the chromosomes took place during meiosis of the pollen mother cells of the ears on the short weak straws sent up later than the ears tested earlier with aceto-carmin.

It has been already emphasised that in nearly all the *Aegilops* × wheat hybrids investigated in these researches, the univalents are divided homotypically in the first or heterotype division, and although

there is generally an uneven distribution of the chromosomes to the two poles, the number is doubtless sometimes equal, the nuclei in interkinesis possessing $2n$ monads. The suspension of the division of the mother cell at the dyad stage would lead to the production of diploid pollen grains, and that this happened in the fertile hybrid discussed, I consider a likely occurrence, the end of meiosis after the first division being probably correlated with the homotypic split at this stage and the depressed vitality of straws late or weak in their development.

Neither this hybrid nor those of Tschermak are completely fertile. Every season in both are found plants which are almost sterile; moreover, individual plants frequently exhibit all intermediates between ears completely sterile and those which bear the full complement of two grains in each spikelet. These differences are doubtless in some degree connected with erratic meiosis and the consequent variability in the number of chromosomes distributed to the single and multiple nuclei of the microspores.

The chromosome content of the gametes, however, is not the only factor governing fertility, for presumably, the proportion of male and female gametes which receive an equal number of chromosomes should be the same in the anthers of all the flowers of a plant. That one flower under such circumstances yields grain and the other none, must be due to other differences than chromosome content of the gametes, one of which appears to be the dehiscence of the anthers, some shedding their pollen freely while others remain closed.

AEGILOPS SPECIES HYBRIDS.

Hybrids were obtained between the species *A. ovata*, *A. cylindrica*, *A. triuncialis* and *A. ventricosa*; all have the same chromosome number ($n = 14$).

(1) *Aegilops cylindrica* Host ♀ × *A. ovata* L. ♂.

This hybrid is of particular interest, for all its morphological characters agree so closely with those of the Linnean species *Aegilops triuncialis* that I am confident that the latter has arisen from the crossing of *A. ovata* and *A. cylindrica*. The hybrid only differs from the wild species in being sterile.

In the earliest prophase examined in this hybrid and its reciprocal, the spireme is discontinuous and consists of a number of extremely fine threads, in which there is no sign of a split. Later, the threads come together in parallel pairs, and filaments clearly double are seen, their

number obviously less than before, although the exact number cannot be determined (Figs. 252, 253). Condensation follows as in Fig. 254, the filaments being thicker and still double. Somewhat later the nucleus contains a number of short, thick, separate chromosomes in which there is no trace of their double nature; some are connected in pairs end to end by a fine thread, and often bent into a loop (Figs. 255-257).

Contraction and condensation of the nucleus and chromosomes continue and a dense knot is ultimately formed, on the margins of which are frequently seen thick chromosomes unabsorbed in it (Fig. 258). At this stage the nuclear membrane disappears.

On the opening out of the knot a spindle is formed, and the metaphase of the heterotype division is established, in which there are 7 to 13 bivalents, most of them of the end-to-end type arranged at the centre of spindle in regular order, the few univalents being distributed irregularly in the equatorial zone or in the cytoplasm off the poles (Figs. 259-261).

Separation of the bivalents is normal, the components moving to opposite poles, but before the completion of the anaphase all the chromosomes become longitudinally split, with the exception of a few of the univalents which may remain undivided for a time, or altogether, at this stage of meiosis (Fig. 262).

Owing to lagging of some of the chromosomes, the number which moves to the poles of the dividing mother cell varies slightly, but in most cases 14 go to each pole.

In the homotype metaphase 28 monad chromosomes are not infrequently found on the equatorial plate, indicating the equal distribution in the previous heterotype division.

In the homotypic anaphase lagging chromosomes are often seen (Figs. 263, 264); some of them fail to become included in the nuclei of the tetrad and appear isolated in the cytoplasm of the microspores (Fig. 265).

From the mother cell the ordinary 4-celled tetrad usually arises, but a few dwarf pollen grains are sometimes formed (Fig. 266).

(2) *Aegilops triuncialis* L. × *A. cylindrica* Host.

In the earliest stage of the prophase observed in this hybrid the diploid number (28) of separate chromosomes was found in many nuclei, along with a deeply staining nucleolus (Fig. 267).

After this stage the nuclear membrane soon disappears, and a compact synizetic knot is seen in the centre of the dense cytoplasm which fills the cell, outlines of stout chromosomes (? bivalents) being sometimes visible (Fig. 268).

On the opening out of the knot both univalents and bivalents appear, the number of each often varying in different cells. In early anaphase 28 chromosomes are readily counted. In some cells 10 to 14 of them are united end to end in pairs, forming 5 to 7 bivalents, in others as many as 12 bivalents are found; these are arranged at the centre of the spindle, the few univalents present being found at the poles. From examination of metaphases and anaphases of the heterotype division it is clear that bivalents both of the end-to-end and side-by-side types are often present; these behave normally, their components separating and moving to opposite poles of the spindle (Figs. 269-272).

In the anaphase of the heterotype division some of the chromosomes lag for a time behind the rest, but all divide longitudinally at this stage of meiosis. The halves of the chromosomes which divide when near the poles remain together, but halves of 6 or 7 which are divided when in the equatorial zone of the cell separate and move to opposite poles of the spindle (Fig. 273).

In most of the cells the homotype division is quite regular, but in a few, lagging chromosomes are present, some of which fail to reach the poles of the spindle in time to become incorporated in the nuclei of the microspores, and remain isolated in the cytoplasm of the latter (Figs. 275-278).

The majority of the mother cells are divided into 4-celled tetrads.

(3) *Aegilops ovata* L. ♀ × *A. ventricosa* Tausch. ♂.

In the early prophase of this hybrid a discontinuous spireme of thin filaments is observed (Fig. 279). Later the filaments become condensed into separate chromosomes (Fig. 280). Then follows synizesis (Fig. 281) from which emerges 28 univalents some of them joined end to end to form bivalents (Fig. 282).

In the metaphase of the heterotype division, the diploid number of chromosomes (28) is readily counted; both univalents and bivalents are seen, the latter, which vary in number from 3 to 7, are of the end-to-end type, with an occasional pair in the form of a ring suggestive of side by side pairing (Fig. 283).

The bivalents arrange themselves on the central fibres of the spindle, and separate in the normal manner to opposite poles; the univalents are scattered somewhat irregularly, with 5 to 7 usually collected near the poles. In the majority of the cells all the chromosomes become divided longitudinally in the anaphase of the heterotype division, but in a few cases from 1 to 7 univalents remain undivided at this stage of meiosis (Fig. 284).

The divided halves of those univalents which have moved into the polar region remain together, but the halves of chromosomes which lag and divide in the equatorial zone, separate from each other and travel to opposite poles (Figs. 285, 286).

The number of chromosomes entering the telophase of the nuclei in the heterotype division is 13 or 14, the occasional deviation from equality being due to lagging chromosomes which sometimes fail to reach the poles in time to become incorporated in either nucleus.

The homotype division was not studied.

(4) *Aegilops cylindrica* Host $\varnothing \times A. ventricosa$ Tausch. σ .

This hybrid, which proved sterile, is remarkable in its resemblance in morphological characters to the wheat *Triticum Spelta*.

In the heterotype metaphase both univalents and bivalents are found. The latter, usually from 5 to 7 in number are chiefly of the parasynthetic type, seen clearly in the polar view of the equatorial plate in Fig. 289. Such bivalents in profile of later metaphases appear as oval rings; a study of numerous cells supports the conclusion that bivalents of the end-to-end type are also formed in this hybrid.

The bivalents occupy the centre of the spindle with the univalents at first distributed mostly in the equatorial zone, but later on the latter are scattered irregularly over the spindle, one or two occasionally off it in the cytoplasm (Figs. 290, 291).

The components of the bivalents separate normally in the anaphase to opposite poles, but the distribution of the univalents is irregular. The number of chromosomes travelling to opposite poles varies from 11 to 17; sometimes 14 go to each, as evidenced by polar views of the homotype metaphase in which 28 chromosomes are found (Fig. 292).

Both the univalents and halves of the bivalents, divide longitudinally in the heterotype division, which is completed with very few lagging chromosomes.

In the homotype division the metaphases are fairly regular, though in some instances one or two of the divided chromosomes lie off the equatorial plate (Fig. 293). Movement from the latter is often irregular and the anaphases show lagging chromosomes, which ultimately give rise to isolated micronuclei (Fig. 294).

The movement of the chromosomes is frequently more rapid in one cell of the dyad than in the other, nuclei in telophase being seen in one while the nucleus of the other is in anaphase (Fig. 295).

Most of the mother cells produce 4-celled tetrads, but, as in the more

irregularly divided cells, the nuclei are rarely of uniform chromosome composition (Figs. 296, 297).

DISCUSSION.

In cytological features the hybrids produced by crossing *A. ovata*, *A. cylindrica*, *A. triuncialis* and *A. ventricosa* among themselves have many points of agreement with the hybrids between the same species and the different races of wheat.

In those hybrids in which the earlier prophases were examined, the spireme consists of extremely fine, unsplit threads which come together in parallel pairs and condense later into short chromosomes exhibiting no sign of their double nature.

As in the *Aegilops* × wheat hybrids a dense synizetic knot is formed, which lies imbedded within dense cytoplasm from which the nuclear membrane has disappeared. On the opening out of the knot, a spindle appears on which the chromosomes arrange themselves, univalents and bivalents being present, the latter more numerous than in the *Aegilops* × wheat hybrids. Both para- and telosyndetic bivalents are seen, the relative numbers being given in the table which follows (p. 244).

Unlike many of the *Aegilops* × wheat hybrids, tripolar and quadri-polar spindles are absent.

In the metaphase of the heterotype division the bivalents are found at the centre of the spindle with the univalents scattered irregularly, some of them near the poles, others in the equatorial zone.

Longitudinal division of the chromosomes takes place at this stage of meiosis and is usually more complete in these than in the *Aegilops* × wheat hybrids.

Lagging for a time is seen in the anaphase, but most of the chromosomes ultimately become included in the nuclei of the divided mother cell. Although there is some variation in the numbers which travel to each of the two poles, these are more frequently equal or nearly equal than in the *Aegilops* × wheat hybrids, and 28 monad chromosomes on the metaphase plate of the succeeding homotypic division are not unusual. This result is no doubt due to the greater number of bivalents in these hybrids.

In interkinesis the two halves of the mother cell are separated by a cell wall, and a typical vesicular nucleus is produced in each.

The homotypic division is fairly regular, and the mother cell is generally divided into a 4-celled tetrad, each cell containing a single nucleus which is, however, variable in chromosome content. In some

cases, lagging chromosomes are found which form micronuclei, more particularly, perhaps, in the hybrid *A. cylindrica* \times *A. ventricosa*. Separation of small portions of cytoplasm from the main mass in the cell and subsequent productions of dwarf microspores are rarely observed.

Hybrid	No. of bivalents	Kind of bivalents
<i>n</i> = 14		
<i>A. cylindrica</i> ♀ \times <i>A. ovata</i> ♂	7-13	Chiefly telo.
<i>A. ovata</i> ♀ \times <i>A. cylindrica</i> ♂	7-13	Chiefly telo.
<i>A. triuncialis</i> ♀ \times <i>A. cylindrica</i> ♂	3-12	5-6 para. rest telo.
<i>A. ovata</i> ♀ \times <i>A. ventricosa</i> ♂	3-7	Chiefly telo.
<i>A. cylindrica</i> ♀ \times <i>A. ventricosa</i> ♂	6-7	Chiefly para.

In regard to the relationships between the species investigated only tentative conclusions can be drawn at this stage of the researches from the cytological features given in the table above; some of the results are, however, worthy of attention.

The greater number of bivalents in these hybrids in comparison with the numbers observed in the *Aegilops* \times wheat hybrids, suggests a closer phylogenetic relationship between the species of *Aegilops* mentioned than that existing between the *Aegilops* species and the wheats.

The presence of 6 or 7 parasyndetic bivalents unaccompanied by any other type in the hybrid *A. cylindrica* \times *A. ventricosa*, is evidence, on the hypothesis proposed in the preceding discussion, that these two species possess 6 or 7 chromosomes which are exactly homologous.

Other remarkable results are the presence of a large number of bivalents in the two hybrids *A. cylindrica* \times *A. ovata* and *A. triuncialis* \times *A. cylindrica*.

In the former there are from 7 to 13 bivalents chiefly of the end-to-end type, which, on the hypothesis put forward, indicate a kind of affinity or homology between the chromosomes of the parents similar to that between chromosomes of different yet closely related species.

The presence, in the second hybrid, of a similar number of bivalents, half of which are of the parasyndetic type, suggests that in *A. triuncialis* 6 or 7 chromosomes are exact homologues of 7 in *A. cylindrica*. This result is highly significant, since it supports the conclusion arrived at by a study of the morphology of the species and their hybrids, that *A. triuncialis* has originated from the hybridisation of *A. cylindrica* and *A. ovata*. Crossing of the hybrid *A. cylindrica* \times *A. ovata* (= *A. triuncialis*) with *A. cylindrica* would bring together 7 exactly homologous chromosomes to form 7 parasyndetic bivalents, leaving the remaining 7 of each parent to conjugate in the end to end manner; a close approximation to this result was observed.

The occurrence of 3 to 7 telosyndetic bivalents in the hybrid *A. ovata* \times *A. ventricosa* indicates a similar but less complete relationship between these two species than that existing between *A. ovata* and *A. cylindrica*.

SUMMARY.

Aegilops \times wheat hybrids.

1. Meiosis was studied in the pollen mother cells of 33 hybrids of *Aegilops ovata*, *A. cylindrica*, *A. triuncialis* and *A. ventricosa* crossed with representatives of different races of wheat and with each other.

2. In those hybrids in which the earliest prophases were examined, single leptotene threads were observed; these become associated in parallel pairs, and the discontinuous spireme is resolved into shorter, thicker chromosomes which where countable are either always diploid or consist of a number of univalents with 1 to 7 bivalents, twice the number of the bivalents plus the univalents equalling the diploid number.

3. Prophases in which clearly defined chromosomes are present are easily confused with diakinesis, especially when bivalents are seen; they are, however, always followed by synizesis.

4. In synizesis the prophase nucleus and its contents contract, and the chromosomes come together and appear as a deeply stained compact mass imbedded in the dense cytoplasm of the cell, the nuclear cavity and membrane being obliterated.

5. From the synizetic knot the chromosomes emerge, the univalents and bivalents taking their place immediately on the spindle which forms at the same time. In many cases the diploid number of univalents is seen, but in most of the hybrids there are one or more bivalents; the latter arrange themselves on the central fibres of the spindle, the univalents being scattered over the spindle with one or two off it in the cytoplasm.

6. The bivalents are of two kinds: (a) telosyndetic bivalents, in which the components are joined end to end, and (b) parasyndetic bivalents, in which the conjugating chromosomes lie side by side. The differences between them are often visible in the prophases immediately before condensation into the synizetic knot, but are most clearly seen in the metaphases of the heterotype division.

7. The components of the telosyndetic bivalents lie in the same straight line: they emerge from synizesis in this position and take their place on the spindle aligned in this manner. The components of the parasyndetic bivalents, are also at first joined end to end, but bend round

later into the shape of a horseshoe, the free ends of which may become joined, the bivalent then assuming the form of an oval link or ring. In some cases the components coalesce and fuse along their entire length, coming to resemble a univalent in form, but of double thickness.

8. It is suggested that parasyndetic bivalents are only formed when exactly homologous univalents meet, the telosyndetic type arising when the conjugating chromosomes are not so closely related. On this hypothesis bivalents of the parasyndetic type would be found in self- or cross-fertilised plants of the same species, telosyndetic bivalents being produced in hybrids between plants which taxonomists consider belong to closely related species or sub-species. In hybrids between distinct species more widely separated the univalents do not pair. Application of this hypothesis to the solution of the problem of relationships between wheats and the species of *Aegilops* investigated is discussed.

9. The constant occurrence of seven parasyndetic bivalents and no other type, in all the hybrids between *A. cylindrica* and wheats of the Bread wheat series, points to the conclusion that seven of the chromosomes in the Bread wheats have been derived from *A. cylindrica*.

In the other *Aegilops* × wheat hybrids, the bivalents are chiefly telosyndetic and not constant, varying between 0 and 5.

10. In the heterotype metaphase the chromosomes are usually scattered irregularly over the spindle, it being extremely rare to find them all arranged on an equatorial plate.

11. In the heterotype anaphase a lagging group of chromosomes is generally seen in the equatorial zone; this feature is observed in hybrids between *Aegilops* species and wheats having the same parental number of chromosomes as well as in those in which the number is unequal. The size of the lagging group varies considerably, but the number in it sometimes suggests that it is composed of chromosomes belonging entirely to one parent, those of the other having separated to the poles.

12. In all the hybrids the chromosomes are homotypically split in the metaphase or anaphase of the first or heterotype division, the split appearing earliest in those cells which possess univalents only; the division is delayed in cells containing bivalents, for it does not begin until the components of the bivalents have separated and are travelling to the poles of the spindle.

13. Only the monads from the divided univalents which are in the equatorial zone when the homotypic division occurs, are separated to opposite poles; in univalents lying near the poles when first divided the halves remain together and enter the same nucleus of the dyad.

14. The number of chromosomes which pass finally to the poles in the heterotype anaphase is extremely variable, and rarely are the numbers equal in the nuclei of the dyad.

15. In interkinesis the nuclei pass into the resting state, and the dyad is divided by a cell wall.

16. The metaphases of the homotype division are usually much more regular than those of the heterotype division, all the chromosomes being frequently collected on the equatorial plate; nevertheless, the movement of the chromosomes from this position in the anaphase is usually very irregular, the number finally distributed to the nuclei of the tetrad varies much, and gametes with equal numbers of chromosomes are very rare.

17. Four-celled tetrads, with a single nucleus in each cell, are common in all the hybrids. The chromosome content of each nucleus is, however, variable owing to the irregular distribution in the homotype anaphase. In addition to the ordinary nucleus, smaller nuclei and isolated chromatic bodies are very frequent in the cells of the tetrads and pollen grains. Twin pollen grains are also developed sometimes. Small pieces of cytoplasm, usually including an aberrant chromosome or two, are often cut off the main mass of one of the cells of the tetrad; these become rounded off to form dwarf microspores.

18. While the spindles in the heterotype division of most hybrids are bipolar, tripolar and occasional quadripolar, spindles are found in some of the hybrids; the departures from the ordinary bipolar arrangement are not considered accidental but probably correlated with the existence in the cell of several groups of non-homologous chromosomes.

Tripolar spindles are rare or absent from hybrids of *A. triuncialis* × wheats, but a few are seen in the hybrids of *A. ovata* and *A. cylindrica* × wheats. In the hybrids of *A. ventricosa* × wheats of the Emmer series they are very numerous, but absent in *A. ventricosa* × *T. monococcum*. Reference is made to these features in the discussion.

19. Six-celled and 8-celled tetrads are produced when tripolar and quadripolar spindles are established in the heterotype division, and in these cases the individual cells of the tetrad are usually of equal size. In the hybrid *A. ovata* ($n = 14$) × *T. monococcum* ($n = 7$) a few pollen mother cells were observed with about 35 univalents: this result appears to have arisen by cytomixis between adjacent mother cells.

20. The hybrid, *A. ovata* × *T. turgidum* var. *iodurum* was fertile, yielding four grains in the ears of short straws which developed late on the F_1 plant. It was similar in morphological characters to other sterile hybrids of *A. ovata* × *T. turgidum* var. *mirabile* and other wheats.

21. In the pollen mother cells in the ears on the first straws of the F_1 plant there were 28 univalents as in all other tetraploid hybrids. In the somatic and mother cells of the F_2 plants 56 univalents were found, suggesting that gametes with the diploid (28) instead of the haploid (14) number were produced in the anthers of the flowers on the weak straws of the F_1 plant.

22. In all the hybrids investigated the chromosomes are divided homotypically in the first or heterotype division, and it is suggested that in this hybrid the division of the mother cell was arrested at the dyad stage, the two cells of the dyad developing into microspores.

23. The mother cells in plants of the fertile hybrid (F_2) are larger than those of the corresponding sterile hybrids.

24. Whether a particular ear is fertile or sterile is not entirely dependent on regular or irregular meiosis, for all the flowers of some of the ears of a plant may produce no grain while some or all of the flowers of other ears on the same plant may be fertile: dehiscence of the anthers and shedding of pollen is essential for grain production, and does not always take place in this hybrid.

25. The cytology of the F_2 plants of this fertile hybrid agrees with that found by Bleier, and confirmed here, in Tschermak's *Aegilotriticum* hybrid *A. ovata* \times *T. dicoccoides*.

Aegilops species hybrids.

26. The cytology of the following hybrids was studied: *A. cylindrica* \times *A. ovata*, *A. triuncialis* \times *A. cylindrica*, *A. ovata* \times *A. ventricosa* and *A. cylindrica* \times *A. ventricosa*.

The hybrid *A. cylindrica* \times *A. ovata* and its reciprocal are morphologically indistinguishable from the species *A. triuncialis* L. and *A. triuncialis* \times *A. cylindrica* is exactly similar in morphological characters to *A. persica* Boiss. (*A. triuncialis* var. *persica*).

All the hybrids, however, are sterile, while the wild species named are fertile.

The hybrid *A. cylindrica* \times *A. ventricosa* very closely resembles *T. Spelta* L.

27. The general cytological features of these hybrids resemble those of the *Aegilops* \times wheat hybrids. The development of the chromosomes in the early prophase proceeds along the same course.

28. The synizetic knot opens out into the metaphase of the heterotype division.

29. The maximum number of univalents in the hybrids *A. ovata* \times *A. cylindrica* and *A. triuncialis* \times *A. cylindrica* is much larger than in the *A. ovata* \times wheat hybrids rising to 12 or 13. With the exception of the hybrid *A. cylindrica* \times wheats of the Bread wheat series all the bivalents in the *Aegilops* \times wheat hybrids are chiefly of the end-to-end type, while in the two *Aegilops* species hybrids named, 5 or 6 parasyndetic bivalents are common, with the rest telosyndetic.

30. The maximum number of bivalents is seven in the hybrids *A. ovata* \times *A. ventricosa* and *A. cylindrica* \times *A. ventricosa*; in the former they are telosyndetic, while in the latter they are mostly of the parasyndetic type.

31. Separation of the bivalents is normal, the components moving to opposite poles, and as in the *Aegilops* \times wheat hybrids, all, or nearly all, of the univalents are longitudinally split in the anaphase of the heterotype division.

32. In all these hybrids there is a greater tendency for the numbers of chromosomes finding their way to the poles in the heterotype division to be equal than is the case in the *Aegilops* \times wheat hybrids; this is doubtless due to the larger number of bivalents in them which separate normally.

I desire to express my thanks to the Research Board of the University, for the grant from the Huntley and Palmer Research Fund, and to Miss K. Goodwin, B.Sc. and Miss B. Pantin, B.Sc. for their valuable assistance with the arduous work of crossing and preparation of sections: without their aid it would not have been possible to deal with the large amount of material examined in these researches.

Note. While this communication was in the press I received from Mr I. Nishiyama a copy of his paper on the genetics and cytology of some *Avena* hybrids (*Jap. Journ. of Genetics*, vol. v. pp. 1-48).

In the F_1 of the hybrid *A. fatua* ($n = 21$) \times *A. sativa* ($n = 21$) 21 parasyndetic bivalents are seen in the heterotype metaphase.

In the F_1 of the hybrid *A. barbata* ($n = 7$) \times *A. strigosa* ($n = 14$) from 7-9 parasyndetic bivalents are observed.

Pairing of the chromosomes is, however, telosyndetic in the hybrids *A. barbata* \times *A. sterilis* and *A. barbata* \times *A. fatua*.

On the hypothesis which I have here advanced as the result of study of the cytology of *Aegilops* \times wheat hybrids, it would appear that all the chromosomes of *A. fatua* and *A. sativa* are exact homologues,

suggesting that the cultivated *A. sativa* has been derived from the wild *A. fatua*.

In the cross *A. barbata* × *A. strigosa*, 7 of the *barbata* chromosomes find exact homologous mates in *A. strigosa*, pointing to the conclusion that *A. strigosa* is an autopolyploid of *A. barbata*.

On the other hand, the presence of telosyndetic bivalents only in the hybrids *A. barbata* × *A. sterilis* and *A. barbata* × *A. strigosa* is suggestive of a definite but somewhat more remote relationship between the two parents of the respective hybrids.

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EXPLANATION OF TEXT-FIGURES.

All the figures are drawn with the aid of Abbe's camera lucida, and are from single sections of permanent preparations with the exception of a small number stained with aceto-carmin (Belling); Zeiss apochromat 2 mm. objective and No. 12 ocular.

Figs. 1-24. F_1 of *Aegilops ovata* \times *Triticum monococcum*.

- Figs. 1-3. Pre-synizetic prophase of heterotype division; 21 univalents in Fig. 3.
 Fig. 4. Synizesis.
 Fig. 5. Opening out of synizetic knot.
 Figs. 6, 7. Heterotype metaphase with telosyndetic bivalents, spindle just forming.
 Figs. 8, 9. Heterotype metaphase with 5 and 3 telosyndetic bivalents respectively.
 Fig. 10. Metaphase; 21 univalents, all except five or six longitudinally split.
 Fig. 11. Heterotype anaphase with 7 univalents in equatorial zone homotypically split; split and undivided univalents at the poles.
 Fig. 12. Late heterotype anaphase with lagging group of chromosomes.
 Fig. 13. Heterotype telophase; two or three undivided univalents at one pole.
 Fig. 14. Homotypic metaphase.
 Fig. 15. Homotypic anaphase, 21 monads in one cell in three groups of 7.
 Figs. 16, 17. Homotypic metaphase and anaphase in halves of a mother cell.
 Fig. 18. Tetrad; chromosome content of each cell different; in two cells are undivided univalents.
 Fig. 19. Tetrad; cells with micronuclei derived from aberrant chromosomes.
 Fig. 20. Triad; heterotype or first division of mother cell following tripolar spindle formation; some undivided univalents in the cells.
 Fig. 21. Hexad following the homotypic division of triad (Fig. 20) resulting in the formation of 6 microspores from the mother cell.
 Figs. 22, 23. Mother cells each containing about 35 univalents (? 21 + 14).
 Fig. 24. Adjacent mother cells showing the beginning of cytotoxicity (?).
 Figs. 22-24 are from the same anther locus.

Figs. 25-41. F_1 of *A. ovata* \times *T. dicoccoides*, and *A. ovata* \times *T. dicoccum*.

- Figs. 25-27. Successive stages of the pre-synizetic prophase of the heterotype division (*A. ovata* \times *T. dicoccum*).
 Fig. 28. Synizesis.
 Figs. 29, 30. Heterotype metaphase (28 univalents). Fig. 29 immediately after opening out of the synizetic knot and the formation of the spindle (*A. ovata* \times *T. dicoccum*).
 Figs. 31, 32. Heterotype metaphase; 1 telosyndetic univalent and 26 univalents (*A. ovata* \times *T. dicoccoides*).
 Fig. 33. Heterotype metaphase; most univalents homotypically split.
 Figs. 34, 35. Heterotype anaphase with lagging groups of divided univalents; some undivided univalents are seen at the poles (*A. ovata* \times *T. dicoccum*).
 Fig. 36. Heterotype telophase; some undivided univalents at the poles (*A. ovata* \times *T. dicoccoides*).
 Figs. 37, 38. Homotypic anaphase with lagging chromosomes (*A. ovata* \times *T. dicoccoides*).
 Fig. 39. Tetrad; cells with micronuclei from aberrant chromosomes.
 Fig. 40. Triad; heterotype division of mother cell following formation of tripolar spindle.
 Fig. 41. Pentad; small microspore split off from one of the tetrad cells; isolated univalents in two of the cells.

Figs. 42-45. F_1 of *A. ovata* \times *T. polonicum*.

- Figs. 42, 43. Heterotype metaphase; 1 telosyndetic bivalent, 26 univalent chromosomes.
 Fig. 44. Heterotype metaphase; all univalents except one longitudinally divided.
 Fig. 45. Heterotype anaphase; two groups of lagging monads from the divided univalents, three or four univalents still undivided.

Figs. 46-49. F_1 of *A. ovata* \times *T. turgidum* var. *mirabile*.

- Fig. 46. Heterotype metaphase, spindle bipolar; 2 telosyndetic bivalents, 24 univalents.
 Fig. 47. Heterotype metaphase, tripolar spindle; 28 univalents.
 Fig. 48. Heterotype anaphase; all univalents divided.
 Fig. 49. Homotypic anaphase, with group of lagging chromosomes.

Figs. 50-59. F_1 of *A. ovata* \times *T. dicoccum* var. *persicum* (Black Persian).

- Fig. 50. Late prophase of heterotype division.
 Fig. 51. Opening out of synizetic knot (heterotype division).
 Fig. 52. Heterotype metaphase; 28 univalents arranged irregularly on spindle.
 Figs. 53, 54. Heterotype metaphases; in Fig. 53 the univalents are scattered irregularly, in Fig. 54 they are chiefly collected in the equatorial zone.
 Fig. 55. Heterotype metaphase; polar view, 26 univalents (the components of a single bivalent above and below the equatorial plate, not included in section).
 Fig. 56. Heterotype metaphase; most of the univalents divided homotypically.
 Fig. 57. Early anaphase of the heterotype division; all univalents divided.
 Figs. 58, 59. Telophase of the heterotype division; nuclei showing evidence of lagging chromosomes.

Figs. 60-76. F_1 of *A. ovata* \times *T. Spelta*.

- Figs. 60, 61. Pre-synizetic prophase of heterotype division.
 Fig. 62. Synizesis, nuclear membrane gone.
 Fig. 63. Opening out of the synizetic knot, spindle developing.
 Fig. 64. Opening out of the synizetic knot, spindle formed.
 Figs. 65, 66. Heterotype metaphases; 35 univalents irregularly distributed in one nucleus, the other with 2 telosyndetic bivalents and 31 univalents.
 Fig. 67. Heterotype metaphase; all the univalents divided homotypically.
 Fig. 68. Heterotype anaphase; 1 or 2 univalents still undivided.
 Fig. 69. Heterotype telophase.
 Fig. 70. Homotype anaphase; lagging group and irregularly arranged chromosomes.
 Fig. 71. Homotype telophase, with lagging chromosomes.
 Figs. 72-74. Irregular homotype divisions; in Fig. 73 three chromosomes off the homotypic spindles have induced the separation of small portions of the cytoplasm from the chief mass in each cell; Fig. 74, later stage of a cell undergoing an irregular division somewhat similar to that in Fig. 73.
 Fig. 75. Heterotype anaphase; mother cell with tripolar spindle.
 Fig. 76. Hexad developed from a cell in which a tripolar spindle was established in the heterotype division.

Figs. 77-84. F_1 of *A. ovata* \times *T. compactum*.

- Fig. 77. Heterotype prophase. Chromosomes separated.
 Fig. 78. Synizesis; nuclear wall gone.
 Figs. 79-81. Heterotype metaphases; in Fig. 79 spindle just formed; in Fig. 80 two telosyndetic bivalents and 31 univalents; in Fig. 81 all except one or two of the univalents homotypically split.

Fig. 82. Heterotype anaphase; the halves (monads) of the divided lagging univalents separating to opposite poles.

Figs. 83, 84. Homotype anaphase; a central group of lagging chromosomes in both cells.

Figs. 85-89. F_1 of *A. ovata* \times *T. vulgare* (Starling).

Figs. 85, 86. Heterotype prophase.

Fig. 87. Synizesis.

Fig. 88. Opening out of the synizetic knot, spindle just forming.

Fig. 89. Heterotype metaphase; 1 telosyndetic bivalent and 33 scattered univalents.

Fig. 89 a. End of heterotype telophase; chromosome content of the two nuclei unequal.

Figs. 90, 91. F_1 of *T. vulgare* \times *A. ovata*.

Fig. 90. Heterotype metaphase; 1 telosyndetic bivalent and 33 scattered univalents.

Fig. 91. Heterotype metaphase; 2 telosyndetic bivalents and 31 scattered univalents.

Figs. 92, 93. F_1 of *A. ovata* \times *T. sphaerococcum*.

Figs. 92, 93. Heterotype metaphases; 3 or 4 telosyndetic bivalents.

Figs. 94-105. F_1 of *A. cylindrica* \times *T. polonicum*.

Figs. 94-97. Presynizetic prophase of the heterotype division.

Fig. 98. Synizesis.

Fig. 99. Opening out of the synizetic knot; 2 telosyndetic bivalents visible.

Fig. 100. Heterotype metaphase; 3 telosyndetic bivalents and 22 scattered univalents.

Fig. 101. Heterotype anaphase; all the univalents divided homotypically.

Fig. 102. Heterotype telophase; chromosome content of the nuclei unequal.

Fig. 103. Interkinesis; single half of mother cell at conclusion of the heterotype division.

Figs. 104, 105. Homotype metaphase and anaphase; in one cell 6 aberrant chromosomes at pole of the spindle.

Figs. 106-110. F_1 of *A. cylindrica* \times *T. dicoccum*.

Figs. 106, 107. Heterotype prophase just before the formation of the synizetic knot; in Fig. 106 are seen 24 chromosomes, 4 of them approximately twice the length of the rest, these doubtless end-to-end bivalents.

Fig. 108. Synizesis.

Fig. 109. Opening out of the synizetic knot; spindle just forming.

Fig. 110. Heterotype metaphase or early anaphase; 28 univalents visible, two coming from the separation of a telosyndetic bivalent.

Figs. 111-122. F_1 of *A. cylindrica* \times *T. turgidum*.

Figs. 111-113. Successive stages of presynizetic prophase; in Fig. 113 are 20 univalents and 4 chromosomes twice the length of the rest, these doubtless end-to-end bivalents.

Fig. 114. Synizesis.

Figs. 115, 116. Opening out of synizetic knot; in Fig. 116 spindle just forming.

Figs. 117, 118. Heterotype metaphases; in Fig. 117 are 28 univalents, in Fig. 118 are 20 univalents and 4 telosyndetic bivalents; this figure is a post-synizetic stage of Fig. 113.

Figs. 119, 120. Heterotype anaphases; in Fig. 119 are 3 undivided univalents at one pole.

Figs. 121, 122. Homotype metaphases.

Figs. 123-134. F_1 of *A. cylindrica* \times *T. compactum*.

Figs. 123, 124. Successive stages of heterotype division; in Fig. 124 are 7 parasyndetic pairs which are seen forming in Fig. 123.

Fig. 125. Synizesis.

Fig. 126. Synizetic knot just before opening out.

Fig. 127. Beginning of the separation of chromosomes and formation of the spindle.

Fig. 128. Heterotype metaphase; polar view showing 7 parasynthetic bivalents on central spindle fibres.

Figs. 129, 130. Heterotype metaphases; scattered univalents and 7 parasynthetic bivalents centrally placed.

Figs. 131, 132. Heterotype anaphases with lagging chromosomes.

Figs. 133, 134. Homotype division; on left, metaphase, on right, anaphase with lagging chromosomes.

Figs. 135-143. F_1 of *A. cylindrica* \times *T. vulgare*.

Figs. 135-138. Heterotype prophases; in Figs. 135, 136 leptoneuma, Fig. 137 pairing of leptotene threads, Fig. 138 separate chromosomes showing double structure.

Fig. 139. Heterotype prophase; ? before the formation of the synizetic knot; some bivalents of parasynthetically paired chromosomes are seen in form of rings.

Figs. 140, 141. Heterotype metaphases; at the centre of the spindle in Fig. 140 are 7 parasynthetic bivalents; at each pole 9 univalents with 3 scattered in equatorial zone; in Fig. 141 the univalents are more irregularly distributed.

Fig. 142. Heterotype metaphase; polar view with the 7 parasynthetic bivalents at centre of the equatorial plate surrounded by 21 univalents.

Fig. 143. Heterotype anaphase; the homotypically divided univalents collected at the poles.

Figs. 144, 145. F_1 of *A. cylindrica* \times *T. Spelta*.

Figs. 144, 145. Heterotype metaphases; 7 bivalents at the centre of the spindle with 21 univalents distributed irregularly.

Figs. 146-152. F_1 of *A. triuncialis* \times *T. durum*.

Fig. 146. Synizesis.

Fig. 147. Opening out of synizetic knot; spindle formed.

Fig. 148. Heterotype metaphase; 6 telosynthetic bivalents and 16 univalents.

Fig. 149. Heterotype metaphase; most of the univalents are homotypically split.

Fig. 150. Heterotype anaphase; monads of 2 univalents lagging.

Fig. 151. Heterotype telophase; nuclei still showing evidence of the movement of the chromosomes to the poles in two groups.

Fig. 152. Homotype division; 4 nuclei of the tetrad; a small portion of the cytoplasm containing a micronucleus has been separated from one of the cells, and will ultimately form a dwarf microspore (cf. Fig. 151).

Figs. 153-164. F_1 of *A. triuncialis* \times *T. dicoccoides*.

Figs. 153-155. Heterotype prophases; presynizetic stages.

Fig. 156. Synizesis.

Figs. 157-161. Heterotype metaphases; Fig. 157 shows 28 univalents soon after emergence from synizesis, and the formation of the spindle; in some of the cells are seen 2 or 3 telosynthetic bivalents separating, with univalents distributed irregularly.

Fig. 162. Heterotype anaphase; univalents divided.

Fig. 163. Heterotype telophase.

Fig. 164. Homotype anaphase; polar view of one cell showing 14 monads on the equatorial plate.

Figs. 165-175. F_1 of *A. triuncialis* \times *T. vulgare*.

Figs. 165, 166. Presynizetic prophases; in Fig. 165 are double filaments resulting from the parallel association of leptotene threads.

Fig. 167. Synizesis.

Fig. 168. Opening out of synizetic knot.

Figs. 169, 170. Heterotype metaphases; one cell with 1, the other with 5 telosyndetic bivalents separating, the univalents irregularly distributed.

Fig. 171. Heterotype anaphase; all the chromosomes divided homotypically.

Fig. 172. Heterotype telophase; nuclei indicating unequal distribution of chromosomes, one with an undivided univalent.

Fig. 173. Divided mother cell (heterotype division), the halves showing 3 or 4 nuclei in each.

Figs. 174, 175. Tetrads; micronuclei in some of the cells.

Figs. 176–185. F_1 of *A. triuncialis* \times *T. Spelta*.

Fig. 176. Presynizetic prophase of the heterotype division.

Fig. 177. Synizesis.

Fig. 178. Opening out of synizetic knot.

Figs. 179–181. Heterotype metaphases; Fig. 179 immediately after opening out of the synizetic knot.

Fig. 182. Heterotype telophase.

Figs. 183–185. Homotype division; in Fig. 183 regular equatorial plate and a single aberrant chromosome; Figs. 184, 185 late anaphase and telophases with lagging chromosomes.

Figs. 186–197a. F_1 of *A. ventricosa* \times *T. monococcum*.

Fig. 186. Presynizetic prophase of heterotype division.

Fig. 187. Synizesis.

Fig. 188. Opening out of synizetic knot and formation of spindle. (? 19 univalents and 1 telosyndetic bivalent.)

Figs. 189–191. Heterotype metaphases; 2–4 telosyndetic bivalents with univalents distributed irregularly; in rare cases some of the bivalents are of the ring form as in Fig. 189.

Fig. 192. Heterotype metaphase; all univalents except 2 homotypically split.

Fig. 193. Heterotype division; late anaphases with 10 monads derived from division of 5 lagging univalents.

Fig. 194. Heterotype telophase.

Figs. 195, 196. Homotype metaphases; profile and polar views, the latter showing dyad (univalent) and monad chromosomes.

Fig. 197. Homotype anaphase with central group of lagging chromosomes.

Fig. 197a. Homotype telophase; a cell with few chromosomes.

Figs. 198–215. F_1 of *A. ventricosa* \times *T. dicoccum*.

Fig. 198. Presynizetic prophase of heterotype division.

Fig. 199. Opening out of synizetic knot.

Fig. 200. Heterotype metaphase; just after formation of the spindle, 28 univalents irregularly scattered over the spindle.

Figs. 201, 202. Heterotype anaphases (bipolar spindles); most of the univalents are homotypically divided in the later stage.

Fig. 203. Heterotype telophase (bipolar spindle); 2 lagging monads.

Fig. 204. Homotype metaphase.

Fig. 205. Homotype anaphase; 2 lagging monads.

Fig. 206. Tetrad developed from mother cell with bipolar spindle in the heterotype division.

Figs. 207–209. Heterotype metaphases (tripolar spindles); in Fig. 209 are 7 univalents collected at each of two poles and 13 at the other pole (1 univalent cut away by the microtome knife).

Fig. 210. Heterotype anaphase (tripolar spindle); all the univalents homotypically divided.

- Fig. 211. Heterotype telophase (tripolar spindle).
 Fig. 212. Homotype division of triad (tripolar spindle) showing 6 nuclei.
 Fig. 213. Hexad; 6 microspores equal in size developed from mother cell in which there was a tripolar spindle in the heterotype division.
 Fig. 214. Heterotype telophase (quadripolar spindle).
 Fig. 215. Octad; 8 microspores equal in size developed from a mother cell in which a quadripolar spindle appeared in the heterotype division.

Figs. 216-220. F_1 of *A. ventricosa* \times *T. dicoccoides*.

- Figs. 216, 217. Heterotype metaphases (bipolar spindles); 28 univalents irregularly distributed.
 Figs. 218, 219. Heterotype metaphases (tripolar spindles); 28 univalents in Fig. 218, at each of two poles 8 univalents have collected 12 at the third pole.
 Fig. 220. Interkinesis (? tripolar spindle); two nuclei at rest with cluster of 7 unaltered chromosomes at one pole and a single aberrant chromosome near.

Figs. 221-231. F_1 of *A. ventricosa* \times *T. turgidum*.

- Figs. 221, 222. Heterotype metaphase (bipolar spindles) 28 univalents in Fig. 222, several showing homotypic split.
 Fig. 223. Heterotype metaphase (bipolar spindle); all the univalents divided homotypically.
 Figs. 224, 225. Heterotype anaphases (bipolar spindles).
 Fig. 226. Interkinesis.
 Fig. 227. Homotype metaphase; 1 chromosome off the equatorial plate.
 Fig. 228. Homotype telophase; a pair of lagging chromosomes joined end to end.
 Fig. 229. Heterotype telophase (tripolar spindle); irregular distribution of chromosomes to the poles.
 Fig. 230. Interkinesis (tripolar spindle); ? following division indicated in Fig. 229.
 Fig. 231. Interkinesis (tripolar spindle).

Figs. 232-238. F_1 of *A. ventricosa* \times *T. polonicum*.

- Fig. 232. Synizesis.
 Fig. 233. Opening out of the synizetic knot and formation of spindle.
 Figs. 234, 235. Heterotype metaphases; spindle just formed; in both cells are 28 univalents.
 Fig. 236. Heterotype metaphase; 28 univalents irregularly distributed.
 Fig. 237. Heterotype telophase; 2 or 3 aberrant chromosomes.
 Fig. 238. Heterotype telophase (tripolar spindle); 7 chromosomes have failed to become included in the nuclei.

Figs. 239-248. F_2 of fertile hybrid *A. ovata* \times *T. turgidum* var. *iodurum*.

- Fig. 239. Heterotype metaphase; polar view, bivalents and univalents. (Belling.)
 Figs. 240, 241. Heterotype metaphases; profile, with bivalents and univalents. (Belling.)
 Fig. 242. Heterotype anaphase; all the chromosomes divided homotypically giving 112 monads. (Belling.)
 Fig. 243. Heterotype telophase; no lagging chromosomes. (Belling.)
 Fig. 244. Homotype metaphase; polar view with 56 chromosomes on the equatorial plate. (Belling.)
 Fig. 245. Homotype metaphase; 3 to 4 univalents off the equatorial plate, some of them dividing.
 Fig. 246. Homotype anaphase; 14 chromosomes lagging in the equatorial zone.
 Fig. 247. Tetrad with micronuclei in all the cells.
 Fig. 248. Immature pollen grain with 3 nuclei.

Figs. 249-251. F_8 of Tschermak's fertile hybrid *A. ovata* \times *T. dicoccoides*.

Fig. 249. Heterotype metaphase; polar view of equatorial plate showing 28 bivalents.

Fig. 250. Heterotype prophase (? diakinesis) showing parasynthetic bivalents in the form of oval rings and some end-to-end bivalents.

Fig. 251. Heterotype prophase; bivalents passing on to the newly formed spindle.

Figs. 252-266. F_1 of *A. cylindrica* \times *A. ovata*.

Fig. 252. Heterotype prophase; fine single leptotene threads.

Fig. 253. Heterotype prophase; leptotene threads in parallel association forming thicker double filaments.

Fig. 254. Heterotype prophase; condensation of united filaments.

Fig. 255. Heterotype prophase; separation of univalent chromosomes (double structures).

Figs. 256, 257. Heterotype prophase; further condensation of the chromosomes.

Fig. 258. Synizesis.

Figs. 259-261. Heterotype metaphases; both para- and telosynthetic bivalents.

Fig. 262. Heterotype anaphase; 4 univalents undivided, 24 divided homotypically.

Fig. 263. Homotype anaphase; lagging monads from a divided univalent in each cell of the dyad.

Fig. 264. Homotype division; lagging chromosomes unabsorbed in the nuclei.

Fig. 265. Tetrad; chromatic bodies in addition to the nuclei in two of the cells.

Fig. 266. Pollen grains of different sizes, two with chromatic masses in addition to nucleus.

Figs. 267-278. F_1 of *A. triuncialis* \times *A. cylindrica*.

Fig. 267. Late prophase with 27 chromosomes, one longer than the rest (an end-to-end bivalent?).

Fig. 268. Synizesis.

Fig. 269. Opening out of synizetic knot and formation of spindle; 28 chromosomes (4 end-to-end bivalents joined by fine threads).

Figs. 270-272. Heterotype metaphases; in Fig. 270 are 11 bivalents (5 parasynthetic, 6 telosynthetic) and 6 scattered univalents; in Figs. 271 and 272 are 11 and 6 bivalents respectively.

Fig. 273. Heterotype anaphase; two groups of 6 or 7 lagging chromosomes.

Fig. 274. Heterotype telophase; 1 unabsorbed monad.

Fig. 275. Homotype metaphase.

Figs. 276, 277. Homotype anaphase with variable number of lagging chromosomes.

Fig. 278. Homotype telophase; one cell with 2 unabsorbed monads.

Figs. 279-288. F_1 of *A. ovata* \times *A. ventricosa*.

Fig. 279. Heterotype prophase; discontinuous spireme of associated threads.

Fig. 280. Heterotype prophase; chromosomes separate.

Fig. 281. Synizesis.

Fig. 282. Opening out of synizetic knot, spindle just forming; 28 chromosomes (one or two end-to-end bivalents).

Fig. 283. Heterotype metaphase; 6 bivalents (1 parasynthetic oval and 5 of the end-to-end type) and 16 scattered univalents. (Belling.)

Figs. 284-286. Heterotype anaphases; in Fig. 284 are 7 to 8 undivided univalents, the rest (20 to 21) divided homotypically; in Fig. 285 the monads from the univalents in the equatorial zone are separating to opposite poles; later stage in Fig. 286 the monads of 6 lagging univalents are still in the equatorial zone. (Belling.)

Fig. 287. Heterotype telophase; no lagging chromosomes.

Fig. 288. Pollen grains; twin and dwarf microspores.

Figs. 289-297. F_1 of *A. cylindrica* \times *A. ventricosa*.

Fig. 289. Heterotype metaphase; polar view of equatorial plate with 6 parasyndetic bivalents and 16 univalents. (Belling.)

Figs. 290, 291. Heterotype metaphases; in Fig. 290 are 7 bivalents (4 parasyndetic, 3 telosyndetic) and 14 univalents distributed irregularly; in Fig. 291 are 4 bivalents (3 parasyndetic) and 20 scattered univalents. (Belling.)

Fig. 292. Homotype metaphase; polar view with 28 monads on the equatorial plate.

Fig. 293. Homotype metaphase; chromosomes divided, some off the equatorial plate.

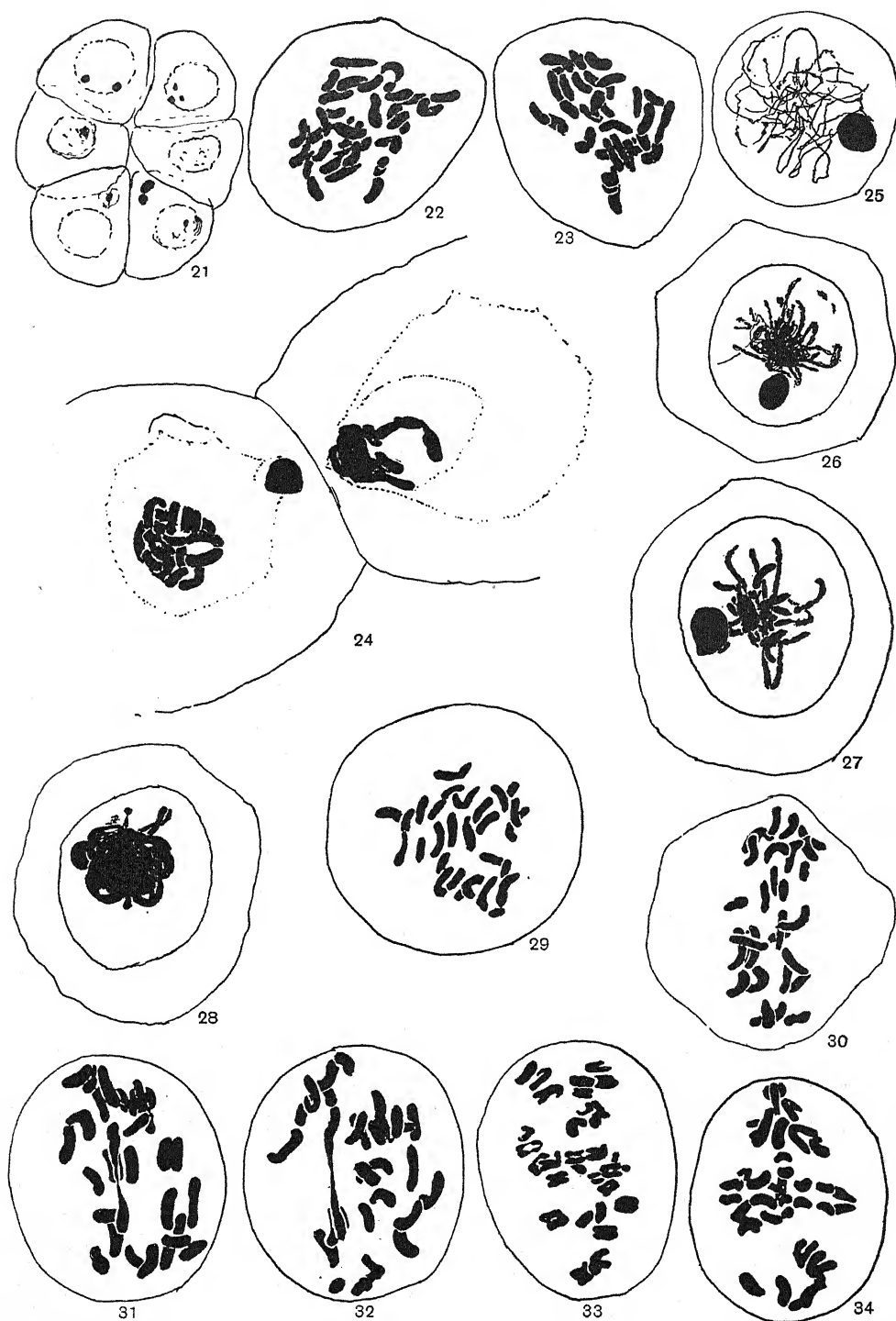
Fig. 294. Homotype telophase; in one of the cells are aberrant monads.

Fig. 295. Homotype division; in one cell the nucleus has reached the telophase, in the other it is in early anaphase.

Fig. 296. Homotype division; in each of the two halves of the dyad are nuclei of irregular form and size and two aberrant chromosomes.

Fig. 297. Tetrad; apparently normal.

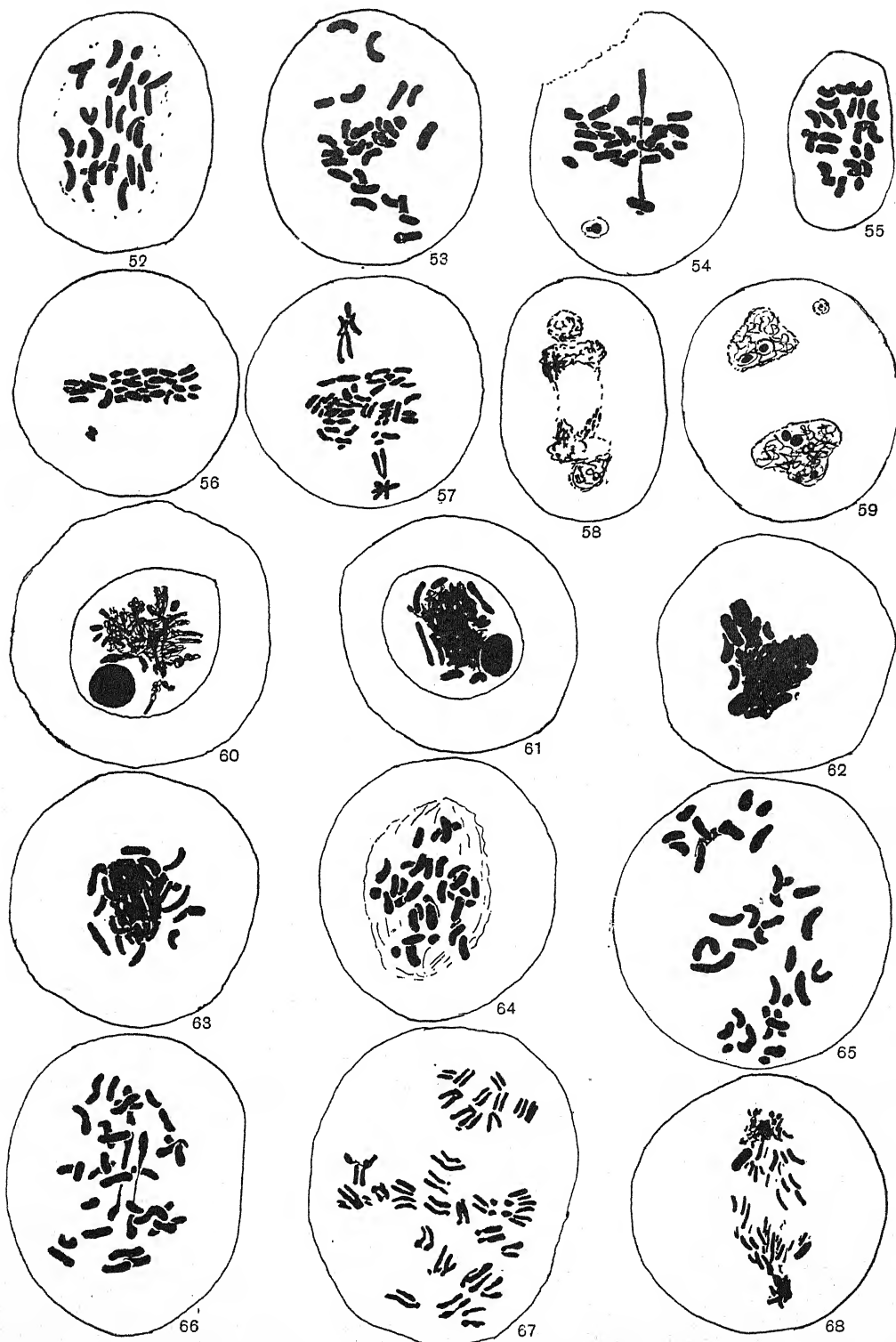
Figs. 1-20, F_1 *A. ovata* \times *T. monococcum*.



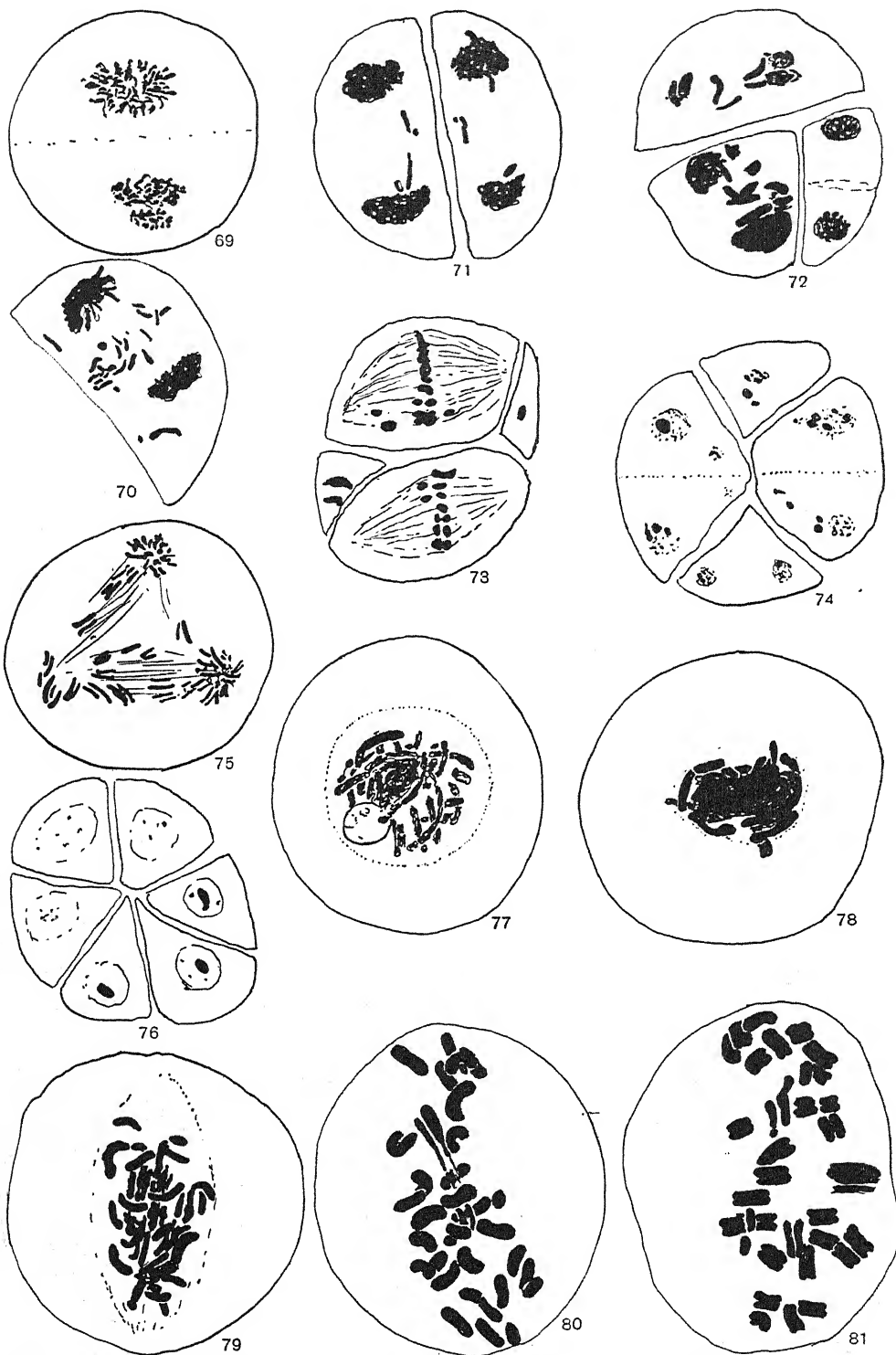
Figs. 21-24, *A. ovata* \times *T. monococcum*; 25-30, 34, *A. ovata* \times *T. dicoccum*; 31-33, *A. ovata* \times *T. dicoccoides*.



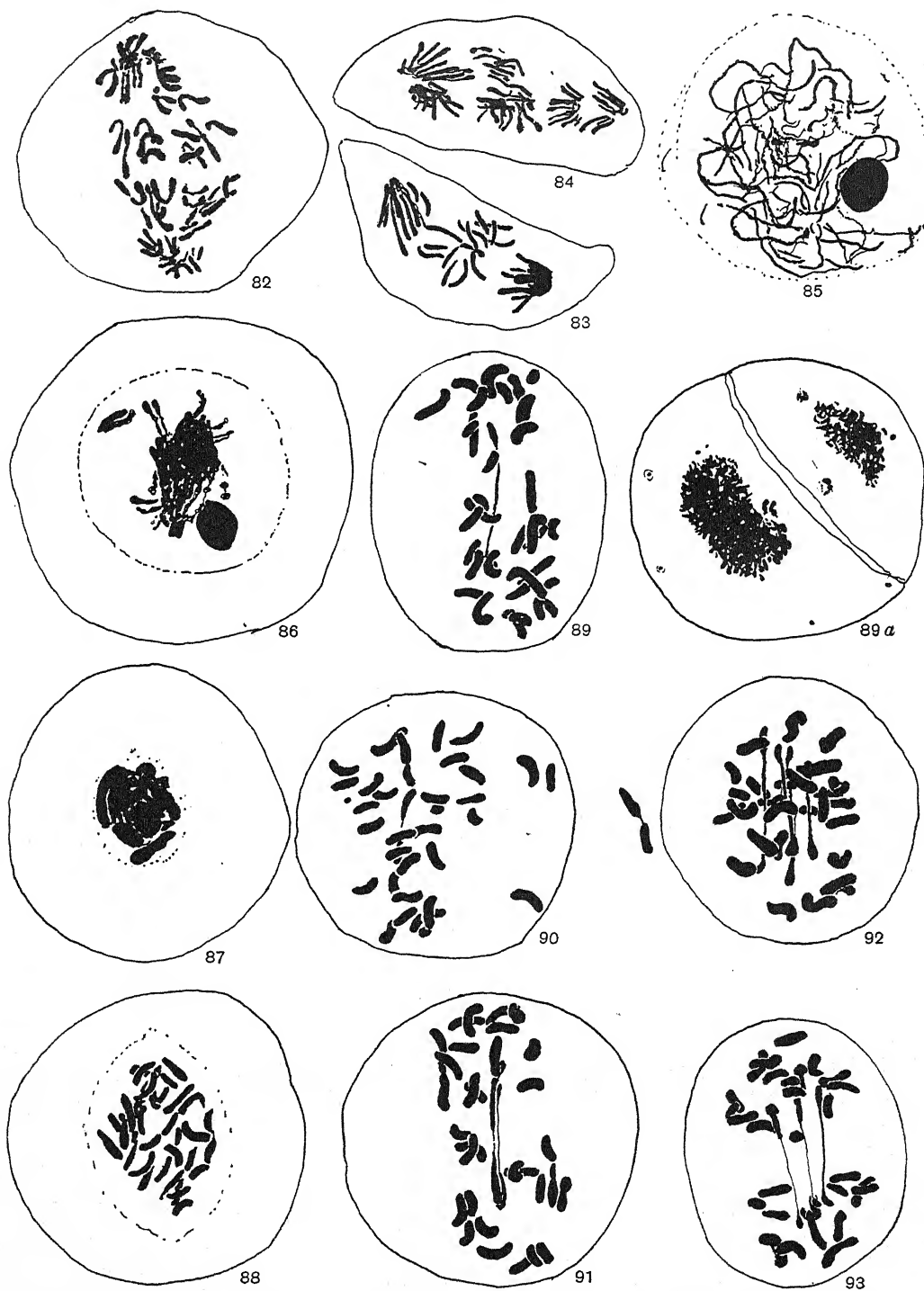
Fig. 35, *A. ovata* \times *T. diococcum*; Figs. 36-41, *A. ovata* \times *T. dicoccoides*; 42-45, *A. ovata* \times *T. polonicum*; 46-49, *A. ovata* \times *T. turgidum*; 50, 51, *A. ovata* \times *T. diococcum* var. *persicum*.



Figs. 52-59, *A. ovata* \times *T. dicoccum* var. *persicum*; 60-68, *A. ovata* \times *T. Spelta*.



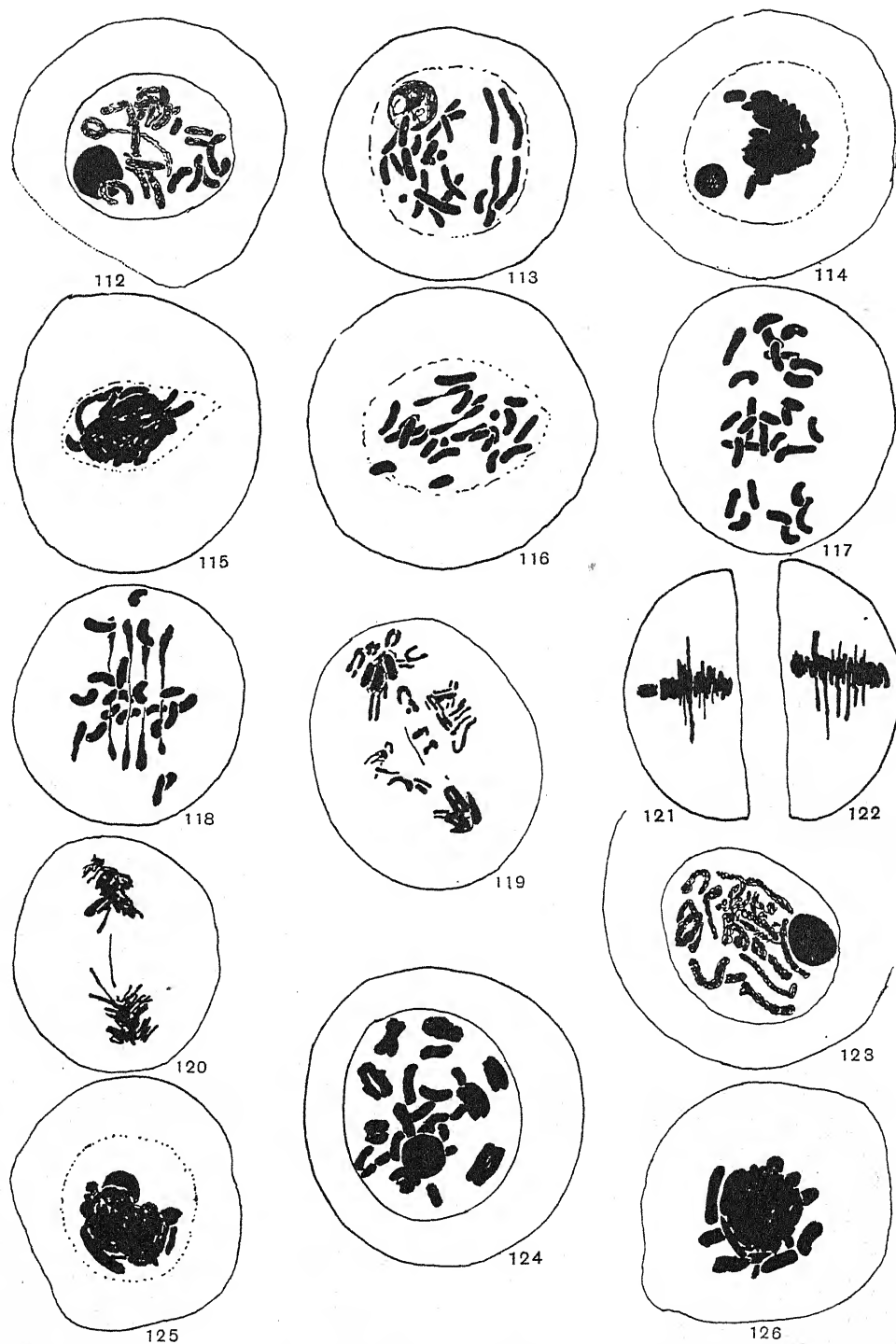
Figs. 69-76, *A. ovata* \times *T. Spelta*; 77-81, *A. ovata* \times *T. compactum*.



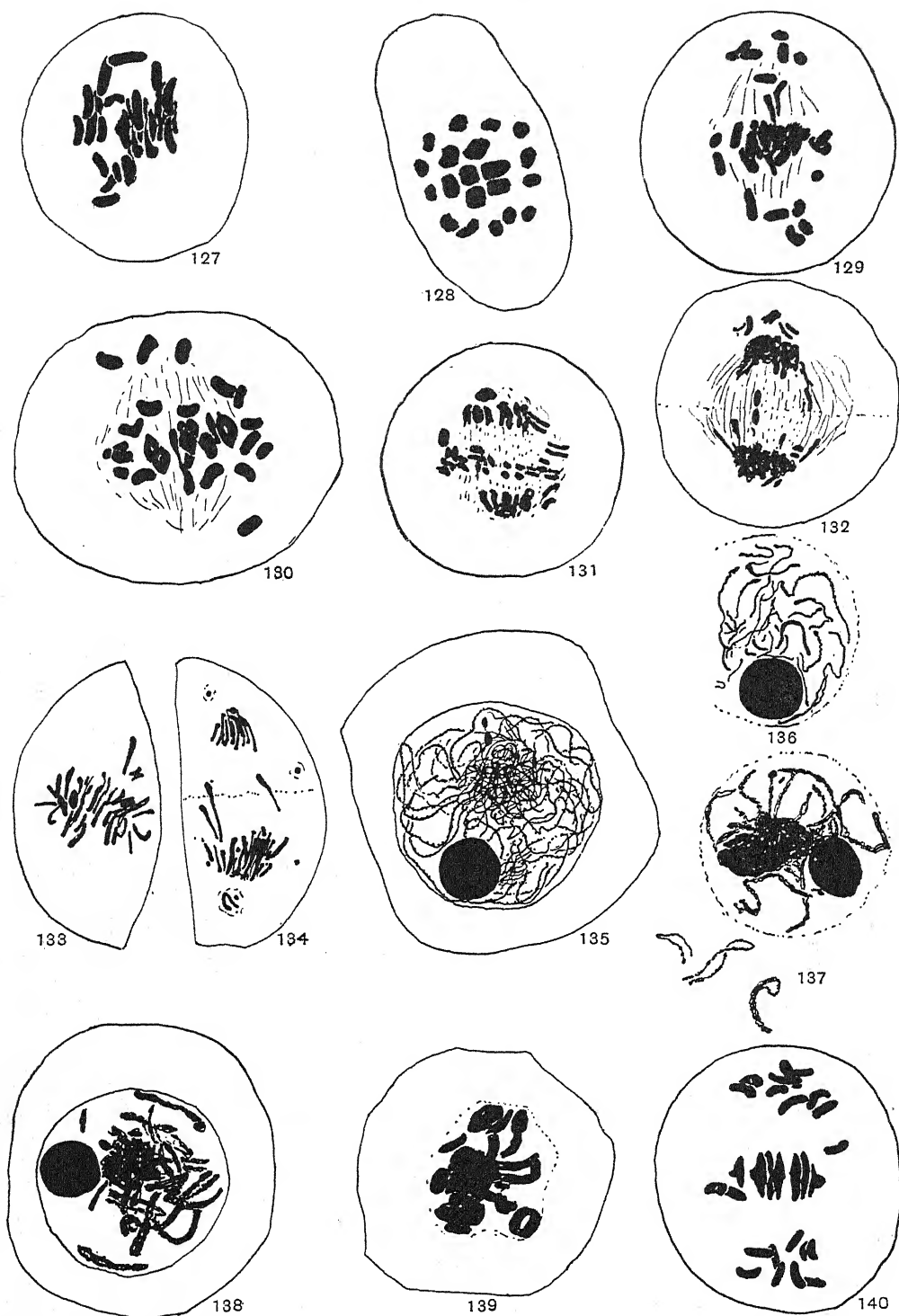
Figs. 82-84, *A. ovata* \times *T. compactum*; 85-91, *A. ovata* \times *T. vulgare*; 92, 93, *A. ovata* \times *T. sphaerococcum*.



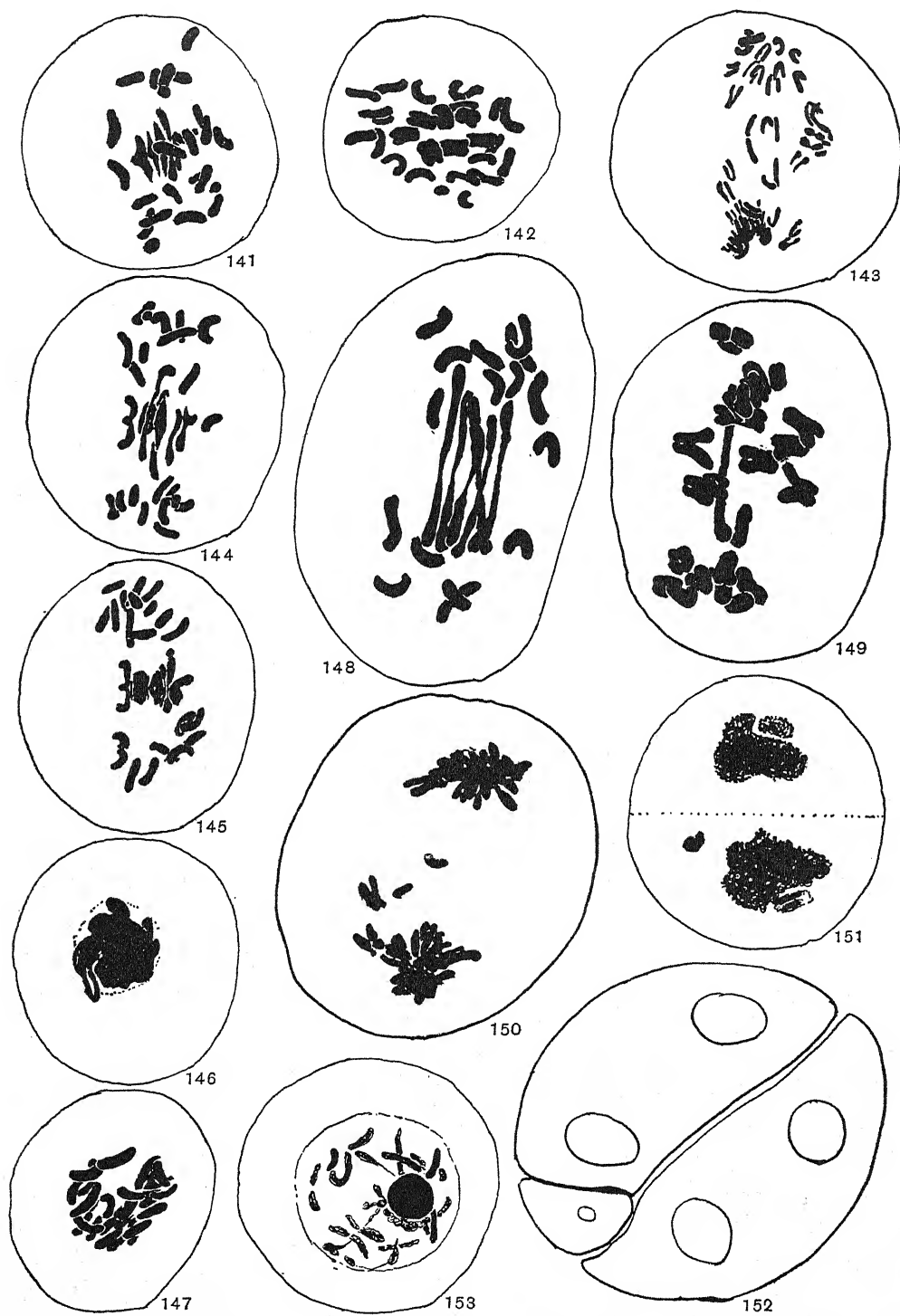
Figs. 94-105, *A. cylindrica* \times *T. polonicum*; 106-110, *A. cylindrica* \times *T. dicoccum*; 111, *A. cylindrica* \times *T. turgidum*.



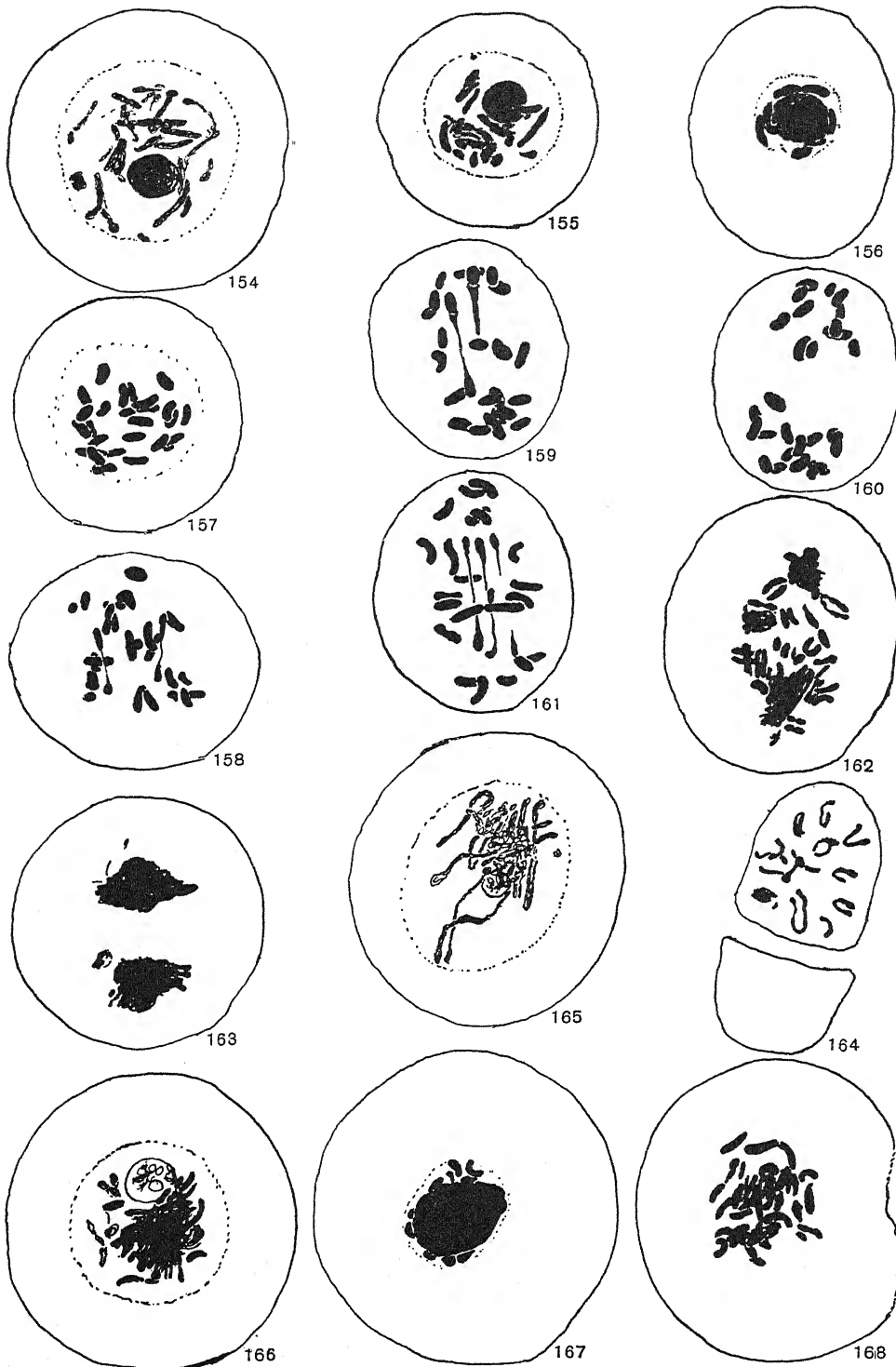
Figs. 112-122, *A. cylindrica* \times *T. turgidum*; 123-126, *A. cylindrica* \times *T. compactum*.



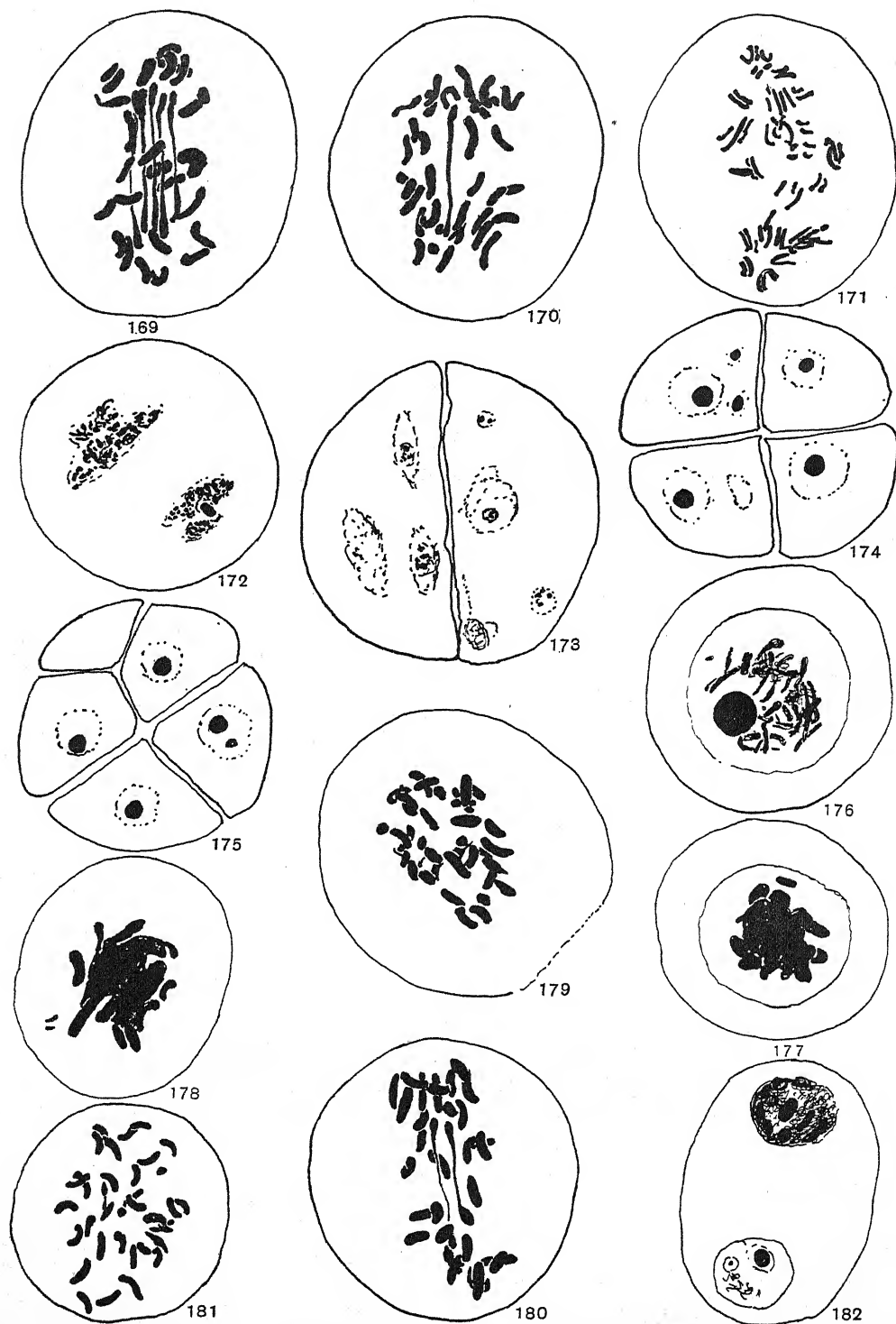
Figs. 127-134, *A. cylindrica* \times *T. compactum*; 135-140, *A. cylindrica* \times *T. vulgare*.



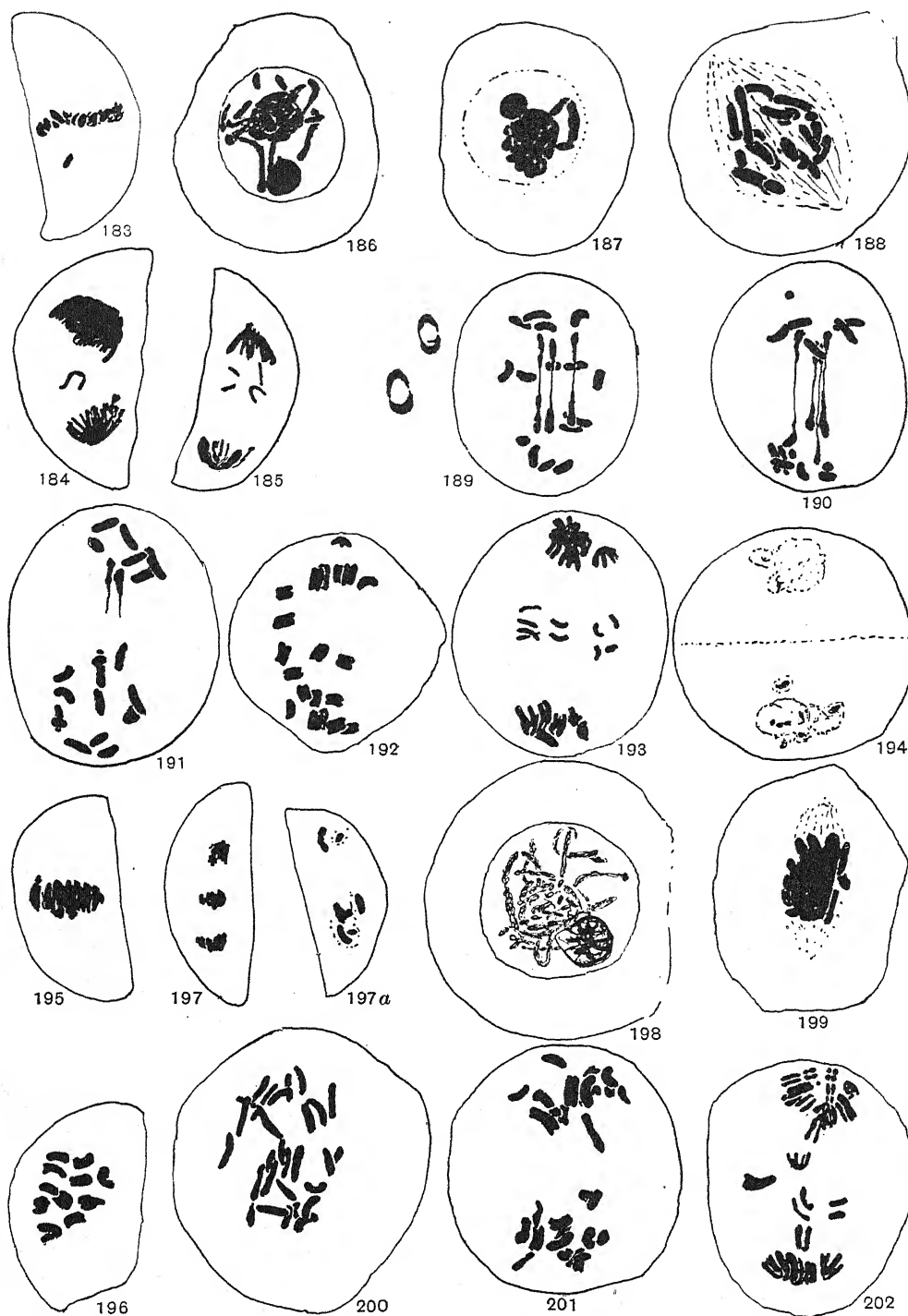
Figs. 141-143, *A. cylindrica* \times *T. vulgare*; 144, 145, *A. cylindrica* \times *T. Spelta*;
 146-152, *A. triuncialis* \times *T. durum*; 153, *A. triuncialis* \times *T. dicoccoides*.



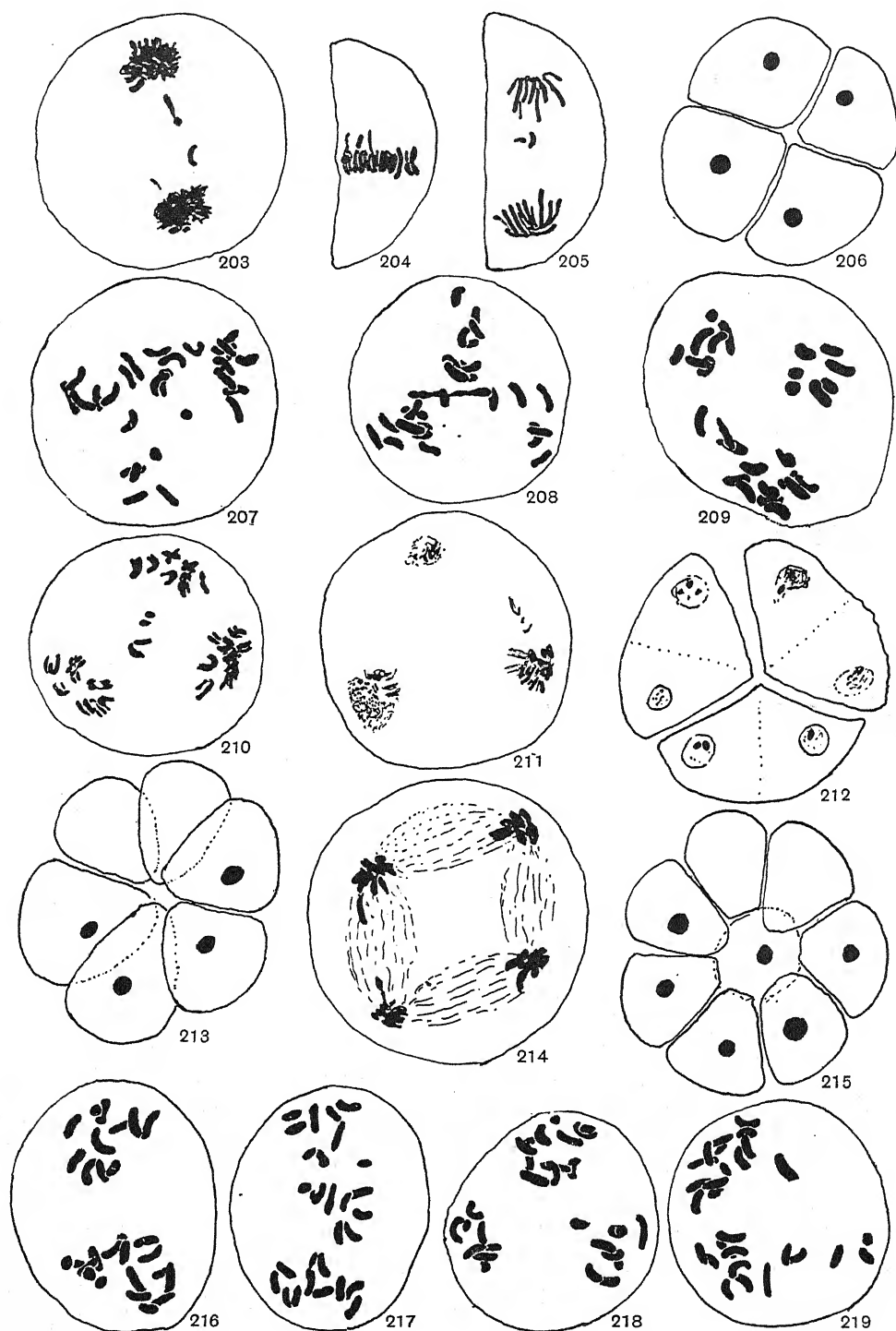
Figs. 154-164, *A. triuncialis* \times *T. dicoccoides*; 165-168, *A. triuncialis* \times *T. vulgare*.



Figs. 169-175, *A. triuncialis* \times *T. vulgare*; 176-182, *A. triuncialis* \times *T. Spelta*.



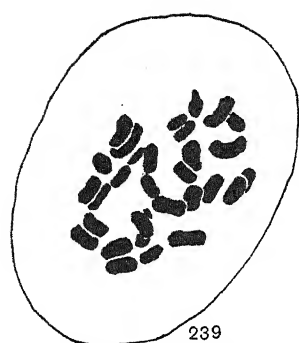
Figs. 183-185, *A. triuncialis* \times *T. Spelta*; 186-197 a, *A. ventricosa* \times *T. monococcum*;
198-202, *A. ventricosa* \times *T. dicoccum*.



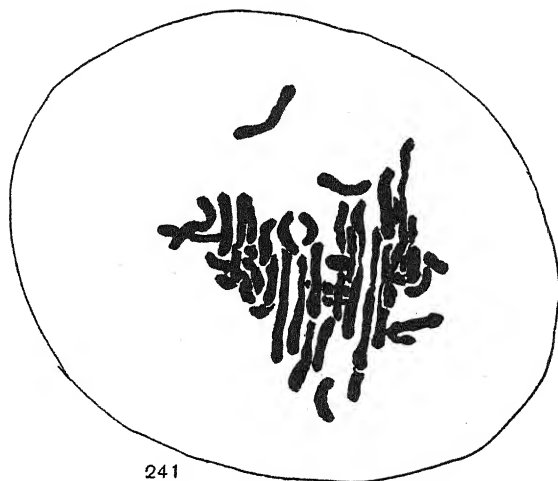
Figs. 203–215, *A. ventricosa* \times *T. dicoccum*; 216–219, *A. ventricosa* \times *T. dicoccoides*.



Fig. 220, *A. ventricosa* \times *T. dicoccoides*; Figs. 221-231, *A. ventricosa* \times *T. turgidum*;
232-238, *A. ventricosa* \times *T. polonicum*.



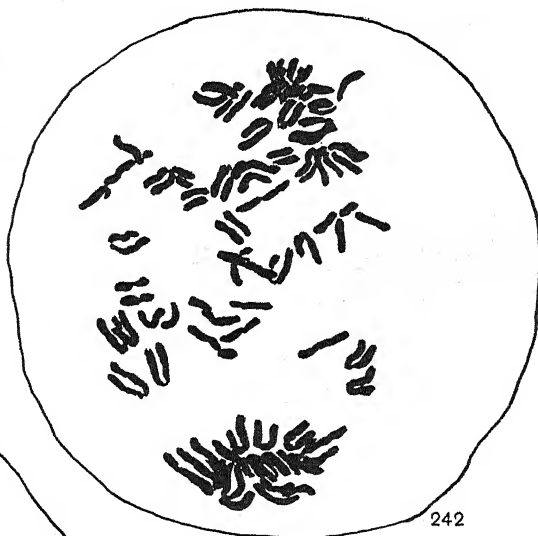
239



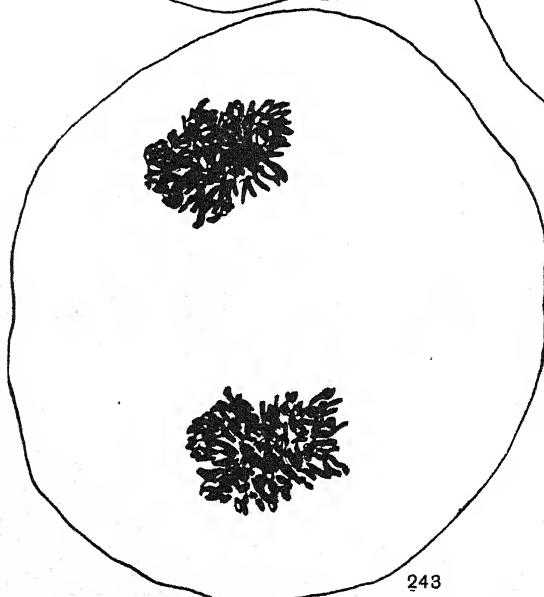
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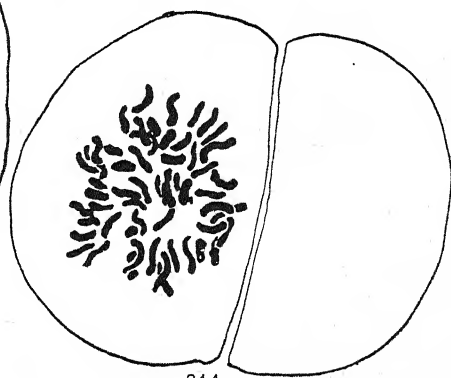
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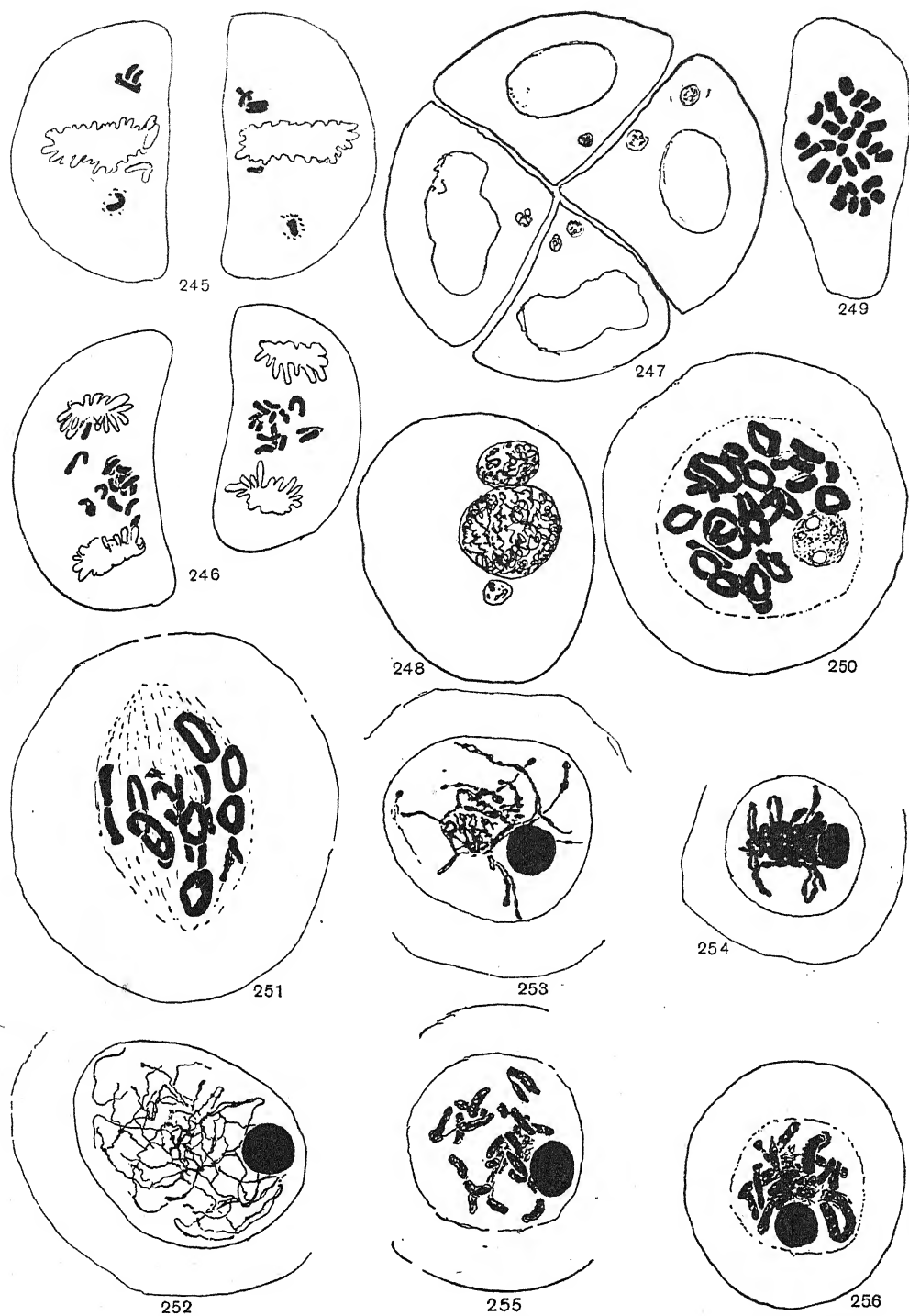


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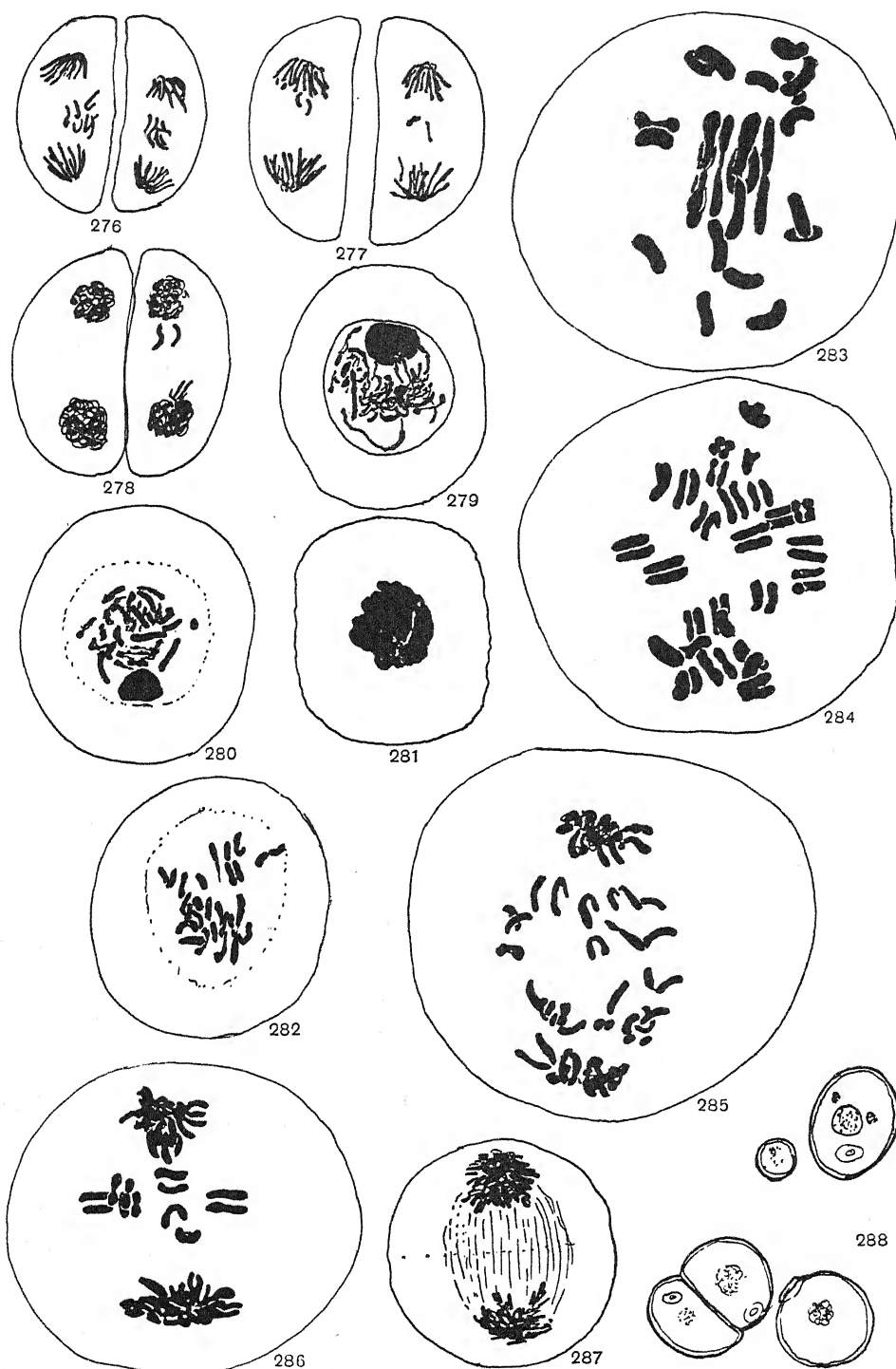
Figs. 239-244, F_2 of fertile hybrid *A. ovata* \times *T. turgidum*.



Figs. 245-248, F_2 of fertile hybrid *A. ovata* \times *T. turgidum*; 249-251, F_3 of Tschermak's fertile hybrid *A. ovata* \times *T. dicoccoides*; 252-256, *A. cylindrica* \times *A. ovata*.



Figs. 257-266, *A. cylindrica* \times *A. ovata*; 267-275, *A. triuncialis* \times *A. cylindrica*.



Figs. 276-278, *A. triuncialis* x *A. cylindrica*; 279-288, *A. ovata* x *A. ventricosa*.

Figs. 289-297, *A. cylindrica* \times *A. ventricosa*.

SHORT SPINE, A NEW RECESSIVE LETHAL IN CATTLE; WITH A COMPARISON OF THE SKELETAL DEFORMITIES IN SHORT-SPINE AND IN AMPUTATED CALVES.

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(Oslo, Norway.)

(With Three Plates.)

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INTRODUCTION.

IN two recent publications (Mohr and Wriedt, 1928; Wriedt and Mohr, 1928) we have called attention to the frequent occurrence of recessive sub-lethal genes in live-stock, and two new representative cases in cattle were then described. In the homozygous condition one of these lethals produces congenital hairlessness (hypotrichosis congenita), while the other causes amputation of all prominent parts (akroteriasis congenita).

It was demonstrated that both these undesirable genes have now become very widely distributed within the Swedish breed of Holstein-Friesians, due to the fact that two very prominent imported sires were heterozygous for the respective genes. In the course of the analysis of these cases no less than three additional recessive lethals in cattle have come to our knowledge. One of these will be described in this paper.

In the publications mentioned above we reviewed the literature on lethal genes in live stock, discussed their occurrence, dissemination and economical importance, and suggested practical measures for preventing their spreading. For literature references and for special considerations on lethals in live stock we may therefore refer to these earlier com-

munications and to a review by Mohr (1929), while a general discussion of the nature and action of lethal genes will be found in an earlier review by the same author (Mohr, 1926).

The lethal gene described in the present paper occurs within the so-called Oplandske mountain breed of cattle in Alvdal, Österdalen, Hedemark Fylke, Norway. For the information on the occurrence of stillborn, malformed calves, which led to the detection of the gene involved, we are indebted to Mr E. Landfastöien, Alvdal. Mr Landfastöien, who is the chairman of the local Bull Association and at whose farm an Association bull, Amor, H. 325, was stationed, had noticed that quite a few stillborn, malformed calves had occurred in matings of this bull to his daughters, or in matings of his sons to his daughters. He had kept careful records of all the matings involving the bull Amor, and kindly placed this valuable material at our disposal. We are also indebted to the district veterinarian, Mr Braastad, and to fylkesagronom Mr Th. Sund for important information.

The investigations on lethal genes in cattle, published in this and in the two above-mentioned communications in this *Journal*, were assisted by a grant from the A./S. Norsk Varekrigsforsikrings Fond.

DESCRIPTION OF THE SHORT-SPINE CALVES.

The abnormal calves are without exception full-term. They are as a rule either stillborn or die immediately after birth. We know of only two cases in which the calf lived one day, and one case in which the calf lived for four days. Suffocation during parturition seems to be the immediate cause of death in many cases (see below).

According to consistent information from several breeders, the abnormal calves are very uniform in appearance, and we may consider the individual to be described as representative in all essential respects (Plate IX, fig. 1).

This calf, a full-term female calf, born 5 April, 1928, and received from Mr E. Landfastöien, was of the ordinary grey colour typical of black individuals at birth. Some white hairs were found around the umbilicus, on the left hind cannon, and around the external genitalia.

The weight was 17.6 kg.

The general appearance of the individual was very odd, due to an extreme shortening of the entire spinal column which contrasts strikingly with the normal length of the extremities (Plate IX, fig. 1). The long legs, the very short neck, the high insertion of the short tail, and a pucker-like prominence in the region of the upper thoracic processus spinosi

combine to produce an elk-like type that may not unlikely account for some of the alleged cow ♀ × elk ♂ hybrids occasionally recorded in the newspapers.

The total length of the individual as measured from the top of the head to the point of the buttock was only 43.5 cm. Measured from the end of the muzzle to the point of the buttock (head outstretched) the length was 66.5 cm. The shortening of the cervical column is very pronounced. In fact, it looks as though the head is fixed to the chest almost without any trace of an intervening neck. The insertion of the tail is abnormally high and slightly asymmetrical (to the right of the median line). The length of the tail was 12 cm.

The transverse diameter of the thorax was very short owing to the abnormally slight curving of the ribs (*pectus carinatus*). The short transverse diameter contrasts strikingly with the long vertical diameter, which was 27 cm. as measured from the top of the above-mentioned pucker-like prominence to the sternum.

The head and all extremities look perfectly normal. The head, from the end of the muzzle to the top of the occipital bone, measured 23 cm.; from the angulus mandibulae to the top of the occipital bone 15.5 cm. The total length of the fore leg (from the olecranon to the point of the hoofs) was 45.5 cm.; the total length of the hind leg (from the upper margin of the patella to the point of the hoofs) 68 cm.

The teeth and the hoofs were normal in every respect.

Except for the changes in the topographical relations of the internal organs caused by the malformations of the vertebral column and of the thorax, the autopsy failed to reveal distinct pathological features. The topographical alterations were most pronounced in the cervical region where the posterior part of the thyroid gland (weight 7 gm.) and the very short trachea were embedded in the thymus (weight 37 gm.), the distal part of which rests on the pericardium. Macroscopically the internal organs, including the glands with internal secretion, presented normal conditions. It should be particularly mentioned that there was no atresia ani, since this anomaly was present in two cases examined by the district veterinarian, Mr Braastad.

Microscopical preparations from the glands with internal secretion were secured, but the post-mortem changes made the material unsatisfactory for examination. So far as could be judged, the grosser histological features were normal.

The parturition of the malformed calves is very difficult, and frequently demands skilled attention. Posterior presentation (in the

majority of cases hind leg presentation) seems to be the rule. Thus, the district veterinarian, Mr Braastad, who has attended five parturitions, found posterior presentation in all these cases. In two cases the difficulties were so great that it was necessary to mutilate the calf. We know of one case in which the cow died during the parturition of a malformed calf, and in another the mother received a permanent distortion in the croup region caused by the very difficult parturition.

HEREDITARY TYPE OF THE MALFORMATION.

As mentioned in the Introduction, the abnormal calves occurred among the offspring of the Association bull Amor, H. 325. In 55 matings to 27 of his own daughters this bull has given 11 short-spine calves of the type described, a result which clearly indicates that we are dealing with a single recessive sub-lethal gene. The expectation is in this case normal and abnormal offspring in the ratio 7 : 1, but the deviation is not greater than those frequently met with in ordinary experiments. Four short-spine calves have also occurred in half-brother \times half-sister matings among sons and daughters of Amor.

Amor was born 30 November, 1917, at the farm of Mr B. Sivilhaug, Alvdaal pr. Auna. From 1 December, 1919 he served as Association bull at the Strand Cattle Breeding Society, Alvdaal pr. Bellingmo. He was normal in every respect, and has on several occasions obtained the second prize at the public shows.

The father of Amor was Glomdölen, H. 198 (see Pedigree, p. 283), born in 1914 at the farm of Mr M. Røsten, who owns one of the most prominent herds of Oplandske mountain cattle. From 1916-24 Glomdölen served as Association bull to the Strømmen Cattle Breeding Society. He has on various occasions obtained the first prize at the public shows. Glomdölen has been frequently mated to his own daughters, and probably not less than 50 calves have been obtained from such matings, all normal. Evidently Glomdölen did not carry the recessive gene here dealt with.

The father of Glomdölen, Magnhilddölen, H. 115, served as Association bull from 1907-14 to the Holmen Cattle Breeding Society, Tynset, and was frequently mated to his own daughters. He was one of the leading sires within the breed, 13 of his sons being used as breeders. The father's father of Glomdölen, the bull Dölen, served as Association bull to the Östby Cattle Breeding Society, Tynset, from 1898-1910. This bull was also frequently mated to his own daughters, since the majority of the cows belonging to this society during his later years were

Amor, H. 325					
Dronning			Glomdølen		
Lykke			Normann	Trygve	Dölen
			Deilig	Litebån	Bull at N. Maehlen
			Magnhilddölen	Dronning	Dölen
				Raudyr	Bull at Ole Grimsbo
				Julegås	Dun and white cow from Ingebret Bergsengseter
				Steifetbull	Bull at Haldo Holen
				Trygve	Cow at Ole Grimsbo
				Rengås	Bull from Ole Grimsbo
					Dun and white cow at Ingebret Bergsengseter
					Litenkoll
					Son of Tronslibull
					Black bull at J. Müller
					Hjertros at J. Müller
					Steifetbull
					Julegås

*Pedigree of the bull
Amor, H. 325.*

his daughters. No abnormal calves are known to have occurred among the offspring of the two last-mentioned bulls. Hence we may safely conclude that they were not heterozygous for the short-spine gene.

The mother of Amor was Dronning, H. 616, born 1906 at the farm of Mr B. Sivilhaug, Alvda. Mr Sivilhaug informs us that no abnormal calves have occurred within his herd. The father of Dronning, the bull Nordmand, born 1897, served from 1900-6 to the Strømmen Cattle Breeding Society. He was frequently mated to his own daughters, and considerable inbreeding has later been carried out with him as a base without any abnormal calves resulting. Of the mother of Dronning, the cow Lykke, we only know that she was born in 1898.

From these data it seems most likely that Amor received the gene for short spine through the cow Lykke, unless—which is, of course, also possible—it arose by mutation in Amor himself, or in his mother, the cow Dronning. At any rate there seems reason for believing that the gene has been detected so early that it will be possible to prevent it from being widely distributed within the breed.

THE SKELETAL DEFORMITIES OF THE SHORT-SPINE CALVES AS
COMPARED WITH THOSE PRESENT IN "AMPUTATED" CALVES.

The following parts of the skeleton of the above-mentioned short-spine calf proved to be perfectly normal, both with regard to size, shape and proportions: the cranium (Plate IX, fig. 3), the os hyoideum, the scapulae (Plate X, fig. 10), the skeleton of the fore leg, the ossa coxae (Plate X, fig. 9), the skeleton of the hind leg.

To the perfect normality of all these parts the general confusion in the make-up of the *vertebral column* (Plate X, figs. 5 and 6) contrasts in a most remarkable way. In fact, the malformations here encountered are so numerous and so irregular that it is impossible to describe them in a satisfactory way.

In the *pars cervicalis*, which normally consists of seven vertebrae, only one, the atlas, presents fairly normal conditions. Although asymmetrical (right half bigger than left), the general type is that of an ordinary atlas.

Distal to the atlas follows an irregularly formed "corpus" vertebrae consisting of three irregular osseous rudiments connected by partly ossifying synchondroses. On the left side this "corpus" is connected with two broad, malformed radices arcus vertebrae which continue into corresponding laminae arcus vertebrae without distinct processus spinosi. On the right side an anterior broad radix arcus and a posterior tiny trace

of a posterior radix arcus vertebrae, fused with the former, connects the corpus with the anterior of the two above-mentioned laminae only. Distal to this confused mass of amalgamated vertebral rudiments follows a corpus vertebrae that is slightly more normal in shape. This corpus is connected on the right side by a relatively normal looking radix arcus, on the left side by a narrow osseous beam, with the posterior of the two laminae just spoken of. Between the two arcus vertebrae there is an articulation on each side, but only on the right side are the facies glenoidales on rudimentary processus articulares. A canalis transversarius is present on the right side of the posterior of these compound cervical vertebrae. On the left side of the anterior of the two a very tiny hole represents the trace of a canalis transversarius. Three vestigial processus transversarii project from the malformed vertebrae in an irregular way.

The pars thoracalis columnae normally consists of thirteen vertebrae. Here only five relatively distinct corpora vertebrae can be recognised. These are very irregular in shape, and some of them are obviously built up of rudiments of adjacent corpora vertebrae which have fused. In addition small cuneiform rudiments of vertebral corpora are in some places intercalated between them from the side and connected with them by synchondroses.

The anterior of these thoracic corpora vertebrae is very abnormal in shape. It is interpreted as belonging to the pars thoracalis, since an indication of a fovea costalis caudalis is present on the left side. But very probably this corpus is composed partly of cervical, partly of thoracic osseous rudiments. This corpus is isolated, the radix arcus vertebrae lacking on both sides. In the corresponding region a broad lamina with a big processus spinosus is connected by means of articulationes with the arcus of the adjacent vertebrae. The form of this processus spinosus suggests that the anterior part of it is not unlikely derived from the posterior cervical processus spinosus.

In addition to this processus only five thoracic processus spinosi are present. But some of these are obviously compound in structure. Thus, in one of them distinct traces of no less than four different processus spinosi may be clearly recognised.

One of the thoracic "vertebrae" lacks a radix arcus on one side, in another the radix arcus is missing on both sides. As regards the processus transversarii and their facies costales, the foveae costales of the corpora vertebrae, etc., the conditions are very confused. This is a direct consequence of the general irregular fusion of neighbouring vertebrae or rudi-

ments of vertebrae. The number of *ribs* is correspondingly reduced. Instead of the normal thirteen ribs only seven costal bones are present on the left, six on the right side. Of these the majority look more or less like normal ribs, while some are irregular bony formations in which the rudiments of two or three fused adjacent costae may be clearly recognised (Plate X, figs. 7 and 8). Corresponding with the abnormal reduced number of the ribs is a radical malformation of the *sternum*. This is very short and thick, and consists of four very irregular osseous fragments joined together by synchondroses.

Malformations analogous to those found in the cervical and thoracic regions are also present in the lumbar region. Instead of the normal six vertebrae lumbales only four compound “vertebrae” are here found. Three of these lack a *radix arcus vertebrae* on one side, and in the fourth, the caudal one, the entire arcus is very rudimentary. The general confusion in the structure of the vertebrae lumbales also applies to their minor parts, the *processus spinosi*, the *processus transversi*, etc.

The sacrum consists of four malformed osseous vertebral rudiments connected by synchondroses, in the middle of which thin osseous discs are found. The two anterior sacral “vertebrae” entirely lack an *arcus vertebrae*, and in the two posterior ones the arcus is very rudimentary. Also in all details the structure of the sacrum is so irregular that an adequate description would be very difficult. Under these circumstances it is very remarkable that the adjacent *os coxae* (Plate X, fig. 9) is absolutely normal.

The vertebrae *coccygeae* are very much reduced in number, fused and so deformed that it is impossible to arrange them in serial order.

Finally it should be mentioned that almost all the compound deformed vertebrae have a *sutura* on each side between the middle part of the *corpus vertebrae* and that part of the *corpus* to which the *radix arcus vertebrae* is attached.

As a result of the radical malformation of the vertebrae the shape of the column as a whole is also abnormal. There is a *lordosis* around the cervico-thoracic junction, a distinct *kyphosis* in the *pars thoracalis* and a very pronounced *lordosis* around the lumbo-sacral junction. Slight *skolioses* are also present: in the *pars cervicalis* (convexity to the left), and in the upper thoracic region (convexity to the right); a very marked *skoliosis* is found around the lumbo-sacral junction (convexity to the right). Finally, from the end of the sacrum the *coccygeal vertebrae* make a sudden bend to the right.

Summing up, we may say that the development of the spinal column

is entirely upset. There is a general tendency to irregular fusion and amalgamation of adjacent rudiments resulting in radical malformations of all the vertebrae and in a very pronounced shortening of the entire vertebral column. Alterations of the same order are also present in the ribs and in the sternum, so that the length of the thorax is correspondingly very much reduced.

It is interesting to notice that the width of the *canalis vertebralis* is unaffected by the radical changes in the form of the vertebrae. Apparently the *medulla spinalis* has nowhere been exposed to pressure. But it is very difficult to understand how it is possible for the spinal nerves to get out from this mess of jammed, malformed vertebrae in any orderly manner.

That the cause of these malformations is selective in its effects is evidenced by the perfect normality of the cranium, the *os hyoideum* and of the skeleton of the extremities, including the scapula and the *ossa coxae*.

It should finally be mentioned that an analogous abnormal calf from the same district was sent in to the Veterinary Institute two years ago. The results of the autopsy (2 February, 1926) were strikingly similar to those presented above in all essential respects. But in addition there was in this case an atresia ani with constipated meconium in the enlarged rectum.

It is of considerable interest to compare the abnormalities of the short-spine calves with those of the amputated calves (see Introduction, p. 279), recently dealt with in this *Journal* (Wriedt and Mohr, 1928). Since the latter paper was published we have received an additional amputated calf from the same district in Sweden. This specimen, which strikingly resembled the former cases in all essential respects¹, was skeletonised, and it was found that the skeleton exhibited very radical malformations of the cranium (particularly the *cranium viscerale*) and of all four extremities, including the scapula and to a less pronounced degree the *ossa coxae*. In striking contrast to these malformations the spinal column is perfectly normal, and this is also true of the sternum and of the ribs, except for the fact that the anterior ribs are less curved than the corresponding ribs of normal calves.

The abnormalities of the cranium and of the extremities of the amputated calves are analogous to those found in the spinal column

¹ The tongue which was normal in the previously described case is here very much reduced in size. The skeletal abnormalities are somewhat more extreme than in the former case.

and the ribs of the short-spine calves, *i.e.* they are due to aplasia or reduction in size and irregular fusion or amalgamation with ankyloses of the adjacent young bony rudiments.

As seen in Plate IX, figs. 4 *a* and *b*, the general appearance of the skull of the amputated calves is very odd. This is mainly due to the extreme reduction or total absence of the bones belonging to the cranium viscerale, while the bones of the neurocranium are only slightly affected, except in those parts which are normally connected with the cranium viscerale.

In the os occipitale the condyli occipitales project in caudal direction. From here the profile line of the calvaria runs in a bluntly rounded curve which contrasts with the more angular outline of the normal calvaria (see Plate IX, figs. 3 and 4 *a*). The ossa parietalia and the os frontale are in the main normal, but the pars nasalis and the processus zygomatici ossis frontalis are bluntly shortened and somewhat defective. In the os sphenoidale the processus pterygoidei are lacking, but otherwise this bone seems to be normal. The sella turcica is of normal size and shape. In the os ethmoidale the lamina papyracea is lacking on both sides so that the somewhat deformed ethmoturbinalia may be seen directly through a defect in the medial orbital wall, to be described below. The ossa temporalia show distinct abnormalities, especially of the pars tympanica. This part, including the bulla ossea, is reduced to mere vestiges in which a tiny hole indicates the very rudimentary meatus acusticus externus. The processus zygomaticus is reduced in size, and of a fossa mandibularis, a tuberculum articulare and a processus articularis only vague traces are found. Two relatively large holes are seen in the squama, dorsal to the radix processus zygomatici (openings of the canalis temporalis?).

Both ossa lacrimalia are lacking. The ossa nasalia are shortened and sharply curved downwards and backwards so that a sudden angular bend (ca. 60°) results. The deformed ossa nasalia deviate somewhat to the left. A shortened, but otherwise relatively normal looking os vomer connects the corpus ossis sphenoidalis with the inside of the bent "nose."

All connections between the above-mentioned neurocranial bones are completely ossified.

Of the bones belonging to the cranium viscerale the ossa zygomatica, the ossa palatina and the ossa pterygoidea are entirely lacking. The maxilla is on both sides reduced to a small irregularly roundish bone in which a single, quite normal looking molar is implanted. The crown of this molar points straight backwards. This maxillary rudiment lies deep

in the orbit and is connected with the side of a very rudimentary os intermaxillare by means of a narrow cartilaginous stalk. The latter is connected with the curved ossa nasalia by a shrivelled cartilago septi nasi on each side of which a narrow anterior meatus nasi is seen. The end of the rudimentary os intermaxillare points downwards and backwards, and deviates slightly to the right. The hiatus caused by the extreme reduction of the maxilla and the absence of an os lacrimale is, along the inside of the curved os nasale, partly filled by a shrivelled plate of cartilaginous tissue in which a small bony rudiment is found on the left side. Surrounded by the free posterior margin of this cartilaginous plate and by the os frontale, the corpus ossis sphenoidalis and the os vomer, a big (3.4–4 cm. \times 1–1.5 cm.) oblongate opening leads directly from the orbit into the narrow nasal cavity on each side. Through this opening the irregular ethmoturbinalia and a cartilaginous septum nasi are directly visible. In Plate IX, fig. 4 *a*, this opening is hidden by the maxillary rudiment.

Every trace of a mandible is lacking, except for three fully developed, normal incisor teeth, the roots of which are glued together by connective tissue in a most disorderly manner (Plate IX, fig. 4 *b*). Of the os hyoideum two relatively well-developed cornua majora are found (Plate XI, fig. 13), while the rest of this bone is represented only by a small osseous fragment that is attached to the left cornu majus by cartilaginous tissue.

The extremities. The skeleton of the right fore limb is reduced to a single slightly curved bone, 7 cm. long, which has a thickening with traces of a fusion of adjacent rudiments 2 cm. above the distal end (Plate XI, fig. 17). The distal end, which is irregularly pointed, was intimately connected with the skin. The skeleton of the left fore limb is even more reduced, forming an angular, 2 cm. + 2 cm. long, osseous rudiment to the distal end of which a tiny roundish bone is attached by aid of connective tissue (Plate XI, fig. 18). Part of the latter small fragment was projecting through the skin.

The scapula is on each side represented by an irregular cartilaginous plate (Plate XI, figs. 14, 15). Only the right scapula contains a 6 cm. \times 3 cm. large osseous lamina in its lower part.

The skeleton of the hind legs consists on each side of a single, very irregular angular bone, ca. 18 cm. long. On the left side (Plate XI, fig. 19), traces of a caput femoris, trochanter major, the completely ankylosed articulatio genus, a short tibia and the end of the os tarsi fibulare are vaguely indicated. On the right side the longest part of the

bone seems mainly to represent the malformed tibia while most of the femur seems to be entirely lacking (Plate XI, fig. 16). On both sides the distal part of the bone consists of an irregular thickening, probably derived from the fused tarsal, metatarsal and phalangeal rudiments. To the somewhat pointed distal end of this thickening is attached a tiny roundish bone on the left side and a well-developed, sharp pointed claw on the right.

The ossa coxae are relatively normal on the left side. On the right side the os ilei is reduced to a very small (5 cm. long) atypical rudiment, while the os ischii and the os pubis are markedly reduced in size. The reduction affects especially the corpus of these bones, and no acetabulum is present on this side of the pelvis. These abnormalities give the pelvis a very asymmetrical form.

To the striking abnormalities of the skull and of the extremities the perfect normality of the vertebral column, including the tail, contrasts in the most remarkable manner (Plate XI, fig. 11). Except for a certain flattening of the anterior ones, the ribs are normal both in number, size and shape. The sternum is normal (Plate XI, fig. 12).

Summing up, we may say that these two different Mendelian recessives produce skeletal abnormalities that are essentially of the same order (aplasia or reduction in size of the developing bones with irregular fusion and ankyloses of the adjacent osseous rudiments). Both genes are strikingly selective in their effect. But from a topographical standpoint the deformities caused by one of these genes are "reciprocal" to those produced by the other, in the sense that the skeletal parts affected in the short-spine calves are normal in the amputated calves, and *vice versa*. This extraordinary situation is well illustrated by the following summary:

	Normal:	Abnormal:
Short-spine calves	Cranium, os hyoideum, scapulae, ossa coxae, skeleton of the extremities	Spinal column, ribs, sternum
Amputated calves	Spinal column, ribs, sternum	Cranium, os hyoideum, scapulae, ossa coxae, skeleton of the extremities

GENERAL CONSIDERATIONS AND DISCUSSION.

There has quite naturally been a tendency to attribute the radical lethal malformations caused by Mendelian genes in mammals to some particular, genetically governed disturbance in the internal secretions.

Though definite evidence is as yet lacking such an hypothesis may seem well founded in cases where the abnormalities affect an entire system, as, for instance, in the different types of bulldog calves described by Crew and by the present authors. Also the congenital hairlessness described by us in this *Journal* (Mohr and Wriedt, 1928) might be conceivably brought about by a mechanism involving some failure in the function of the thyroid.

But in cases like the one here dealt with, where the malformations affect in a strictly selective way the spinal column and the costae and sternum only, leaving the rest of the skeleton entirely uninfluenced, it seems impossible to account for the facts met with by any simple mechanism involving disturbances in the internal secretions. This becomes still more obvious when the skeletal abnormalities of the short-spine calves are compared with the analogous but "reciprocal" malformations encountered in the amputated calves.

In such cases any hypothesis involving disturbances in the internal secretions will doubtless have to be combined with Goldschmidt's conception of "Abgestimmte Reaktionsabläufe, die von Mendelnden Genen abhängig sind" in order to be at all conceivable. But even then we are admittedly very far from having any real idea as to how such strictly selective changes may be brought about. And why is there in the case of the amputated calves a constant correlation between the malformations of the cranium viscerale and the analogous malformations of the extremities? To our knowledge no known embryological facts can account for this peculiar situation. We have also searched in vain for some clue in the experimental results of the "Entwicklungsmechanik." We are apparently confronted with genetically governed developmental principles, the nature of which is as yet unknown.

We have now discovered 6 clear cases of lethals in cattle, 5 of them during the past 2 years. In all, 8 cases are known in cattle, and we have investigations under way which make it probable that this number will soon have to be raised to 10. This may seem a surprisingly high number when it is remembered that the total number of lethals detected in other mammals is 11, 3 being the highest number in any one species.

To conclude from this that lethal mutations occur more frequently in cattle than in other mammals would be entirely wrong. The reason for the relative frequency of lethals in cattle is obviously to be sought in the particular breeding habits in this material. In cattle every mature female is used as breeder and gives regularly one calf a year. The value of the offspring is relatively high. Consequently, stillborn or malformed

offspring are much more likely to attract attention than in sheep or swine. In the latter dead or malformed offspring are simply thrown away without further notice.

In the other valuable and uniparous domesticated animal, the horse, only a limited number of the females are used as breeders, and the fertility is also here much lower than in cattle. Of mares that are used for breeding hardly more than 50 per cent. deliver offspring.

In view of the frequent occurrence of sub-lethal genes in cattle, it seems surprising that relatively few such genes have as yet been discovered in the other mammals, especially in rodents which have been used so extensively in experimental work. In rats, for instance, not a single lethal is so far known (cf. Mohr, 1929).

This is probably due to the fact that in rodents the mother is apt to eat stillborn or weak offspring together with the after-birth, so that, unless special precautions are taken, individuals that are homozygous for a sub-lethal gene are likely to escape detection. This assumption receives strong support from the fact that in the case of dominant white spotting in mice, discovered by Little in 1915, he himself concluded that it was absolutely lethal in a homozygous condition, quite like the dominant gene for yellow body colour. Not until 1923 was it discovered by Detlefsen, and independently by de Aberle, that the gene is only sub-lethal when homozygous. The majority of the homozygotes are born alive as somewhat smaller, weak individuals suffering from a particular type of congenital anaemia which leads to death either at birth or in the course of the first week (see de Aberle, 1925, 1927).

In a former paper (Wriedt and Mohr, 1928) we have suggested the following method for the prevention of the spreading of sub-lethal recessive genes in cattle, viz. that bulls selected as breeders should be required to have given 20 normal calves in matings to their own daughters before being allowed to be used on a larger scale.

In districts where milk production is intensive this test may be carried out without very great difficulty, since under these conditions a large number of heifers are ordinarily reared. It may, of course, happen that the number of daughters will be insufficient, but such cases will doubtless be rare in cattle-breeding associations and in larger herds where bulls are reared for breeding purposes. Under such conditions there will practically always be reared an ample number of heifers also.

Even under more primitive conditions it should be possible to carry out the above test. Where milk production is low, the cows getting only hay and no cake during the winter, they are not so soon worn out, and

will accordingly be kept until they are older than is the case in typical milk-producing districts. But even under such very primitive conditions at least 1 heifer calf must be reared to every 7 cows. In a herd of 80 cows this makes 12 heifers a year. Thus, in the course of 2 years, a sufficient number of daughters may be at hand for the test of the bull in question. A bull is generally used for breeding when 2 years old. He will accordingly be 4 years old when the second year's output of daughters has been obtained. These cows will calve when they are $2\frac{1}{2}$ to 3 years old. Hence the bull may have been properly tested when he is $6\frac{1}{2}$ to 7 years old. In our paper, quoted above, we have demonstrated that no less than 45 per cent. of the Norwegian Telemark bulls registered during the years 1910-20 were 6 years old, or older. Hence it is not impracticable to require that only bulls that have been tested in the above-mentioned way should be permitted to serve as breeders on a large scale.

As regards the statistical efficiency of the method suggested, the following may be mentioned¹. In the cross $AA \times Aa$ the probability of obtaining AA is $= 1/2$, and of obtaining $Aa = 1/2$. In any 20 cows derived from the mating $AA \times Aa$ all combinations from 20 AA to 20 Aa may occur, totalling 21 possible combinations. The probability for each of these combinations may be calculated from the binomial formula. In the mating $AA \times Aa$ the probability of not obtaining aa , *i.e.* for 0 aa , equals 1, while in the mating $Aa \times Aa$ the probability for 0 aa equals $3/4$. When this is taken into account, the expression for the probability of not obtaining aa (*i.e.* of 0 aa) in the test discussed, may, when the calculation is carried out, be reduced to:

$$(1/2)^{20} (1 + 3/4)^{20} = (7/8)^{20} = 0.0692.$$

Hence the measure of safety of the method is ca. 1 : 15, *i.e.* only in 1 out of ca. 15 cases may a bull that is heterozygous for a recessive sub-lethal gene be expected to escape detection even though tested in matings to 20 daughters. Thus, the method is fully satisfactory for practical purposes. In this connection it should also be remembered that in practice it may happen that some of the daughters to which the bull is mated may have received the same sub-lethal, for which the bull is heterozygous, from their mother. Under these conditions the chances of unmasking the heterozygosity of a bull are clearly greater than in the above calculation, where all the cows from which the daughters are derived are considered free from the sub-lethal gene. The measure of

¹ For valuable advice in this connection we are indebted to Mr E. Gjermoe and Mr Fr. Lange-Nielsen.

safety of the method, viz. 1 : 15, applies in other words to the *least* favourable situation for the detection of the gene.

We have been asked why we have not recommended testing the bulls by mating them to cows already proved to be heterozygous. Geneticists working with a rapidly propagating material would clearly be inclined to prefer this method, since it shortens the testing period. But this method can only be used where it is a question of testing for a particular sub-lethal gene, which is known beforehand to be present within the district or herd in question.

In matings of a heterozygous, *Aa*, bull to cows which are known to be heterozygous, *Aa*, the probability of obtaining 0 *aa* is $\frac{3}{4}$. Hence the number of matings to heterozygous cows demanded in order to equal in effectiveness the 20 test-matings to own daughters may be calculated by aid of the equation $(\frac{3}{4})^x = 0.0692$, i.e. $x = 9.3$. This means that 9 to 10 matings to known heterozygous cows equal in effectiveness 20 matings to own daughters.

Even in a large herd it will very rarely happen that 9 to 10 cows, that have already proved to be heterozygous, are available. And to buy such cows from other herds as test individuals would, for various practical reasons, be entirely out of the question. But, as mentioned in our former paper, it is clearly advisable, where heterozygous cows are at hand, to utilise them for testing and, by combining the two methods, to shorten the testing period. One mating to a known heterozygous cow equals 2 matings to own daughters in effectiveness. Occasionally, when it is a question of testing for a particular sub-lethal gene, it may also be advisable to test a son of a known heterozygous bull by mating him to his own half-sisters. This test will be identical with the test to own daughters and require the same number of test matings.

We are, however, of the opinion that the matings to own daughters should be the main method, not only for practical reasons, but because it is the more general in scope and likely to lead to valuable information in other respects. For by the time a tested bull has given 20 calves in matings to his own daughters, these latter will have reached an age that will enable us to obtain valuable information on the genotype of the bull in other, economically important respects, such as milking capacity, butter-fat percentage, etc.

The method of mating association bulls to own daughters is already used to a considerable extent in different districts of our country. For different steps in the practical application of the method we may refer to our former paper (Wriedt and Mohr, 1928).

SUMMARY.

A new recessive lethal gene, occurring within the Oplandske mountain breed in Norway, is described. In a homozygous condition this gene produces a very extreme shortening of the vertebral column and of the thorax. The very short neck and thorax, a pucker-like prominence of the upper thoracic processus spinosi and the high insertion of the very short tail combine, with the normal head and the long, normal legs, to give the homozygous calves a most remarkable elk-like appearance.

These calves are full-term and, except for the shortening, of normal size. The parturition is difficult owing to the frequent posterior presentation, and as a rule demands skilled attention. Except for the changes in topographical relations, the internal organs seem to be normal. Atresia ani was present in some cases. The malformed calves die either during or immediately after birth.

Such calves occurred among the offspring of a particular Association bull, Amor, H. 325, which in 55 matings to 27 of his own daughters gave 11 short-spine calves. Four short-spine calves have also occurred in half-brother \times half-sister matings among the sons and daughters of Amor. Hence we are dealing with a single recessive sub-lethal gene. Pedigree investigations are presented which indicate that the gene has not so far attained a wide distribution, so that it should be possible to check its further spreading.

The skeleton of a short-spine calf is described in detail. The shortening of the vertebral column is due to aplasia or reduction in size with irregular fusion and amalgamation of adjacent vertebral rudiments during development. Thus, a reduced number of malformed compound "vertebrae" are formed, in which traces of fused or intercalated vertebral rudiments may be more or less clearly recognised. Corresponding to the very marked shortening of the thoracic part of the vertebral column, the ribs and sternum have undergone analogous changes. Thus, instead of the normal thirteen ribs, only seven costal bones are present on the left, six on the right side. In some of these traces of two or three adjacent ribs may be recognised. In striking contrast to these malformations, the cranium, the hyoid and the skeleton of the extremities, including the scapulae and the coxae, present perfectly normal conditions.

For comparison the skeleton of one of the amputated calves recently dealt with in this *Journal* (Wriedt and Mohr, 1928) is described in detail. The skeletal abnormalities in the amputated calves are found to be analogous to those present in the short-spine calves, viz. aplasia or re-

duction in size with amalgamation and fusion of adjacent osseous rudiments. But here the changes affect more especially the maxillary, mandibular and hyoid regions of the skull together with the scapulae, the coxae and the skeleton of the free extremities, while the spinal column, ribs and sternum present normal conditions.

Thus, two different recessive, sub-lethal genes produce skeletal abnormalities that are essentially of the same order, though both genes are strikingly selective in their effect. From a topographical standpoint the deformities caused by one of these genes are "reciprocal" to those produced by the other.

This remarkable situation is discussed.

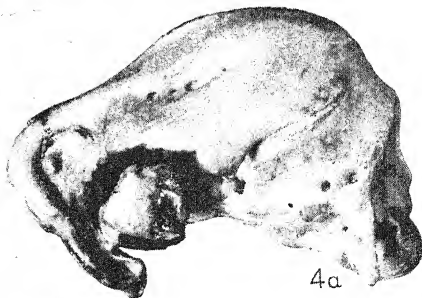
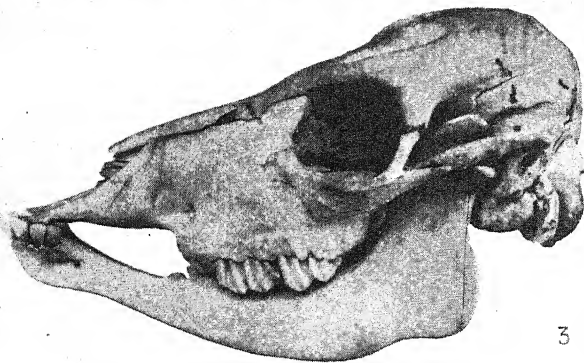
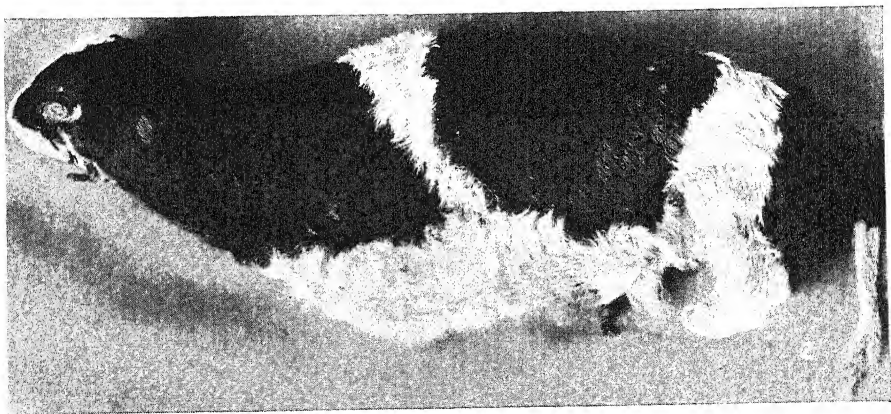
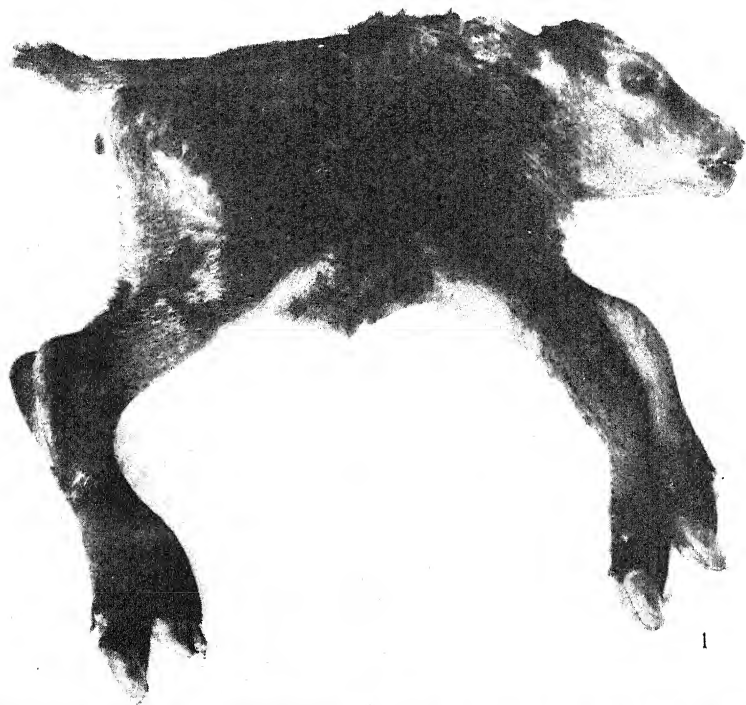
Attention is called to the fact that we now know no less than eight (possibly ten) different sub-lethal genes in cattle, while the total number of lethals known in other mammals is eleven, three being the highest number within one species. Reasons for this are presented.

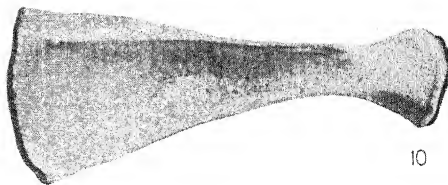
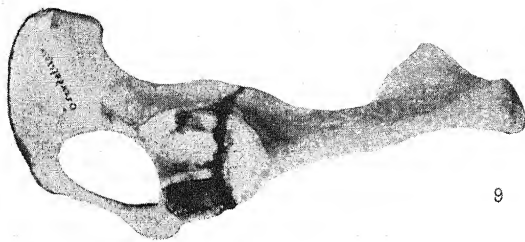
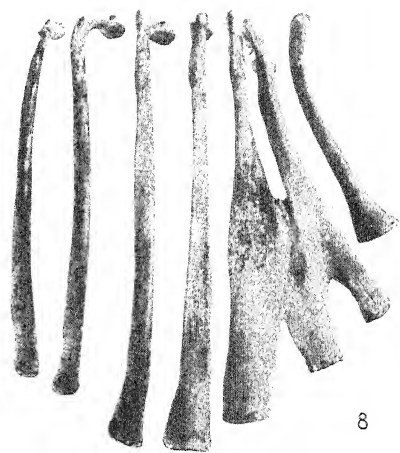
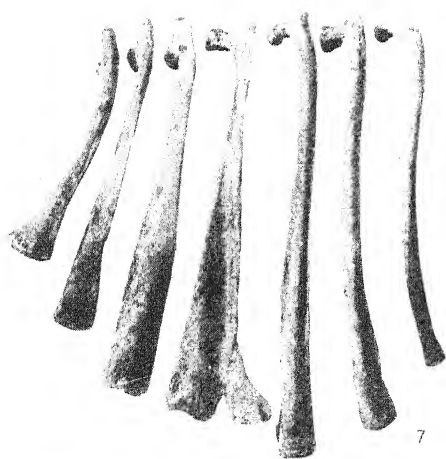
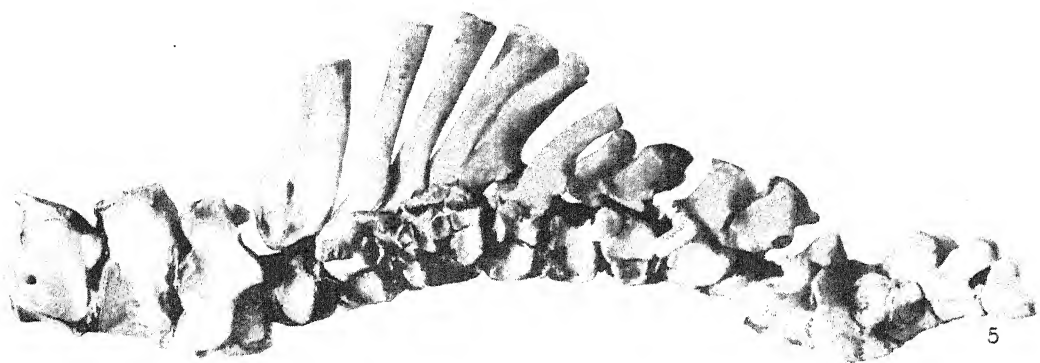
The earlier suggested method for preventing the spreading of sub-lethal recessives, viz. the testing of the bulls by 20 matings to own daughters, is further discussed. It is demonstrated that the statistical measure of safety of this method is ca. 15 : 1, i.e. only in 1 out of ca. 15 cases will a heterozygous bull escape detection. The supplementary method of testing the bulls by matings to known heterozygous cows is also considered. It is shown that 9 to 10 matings to known heterozygous cows equal in effectiveness 20 matings to own daughters, i.e. 1 test-mating to known heterozygous cow equals 2 test-matings to own daughters.

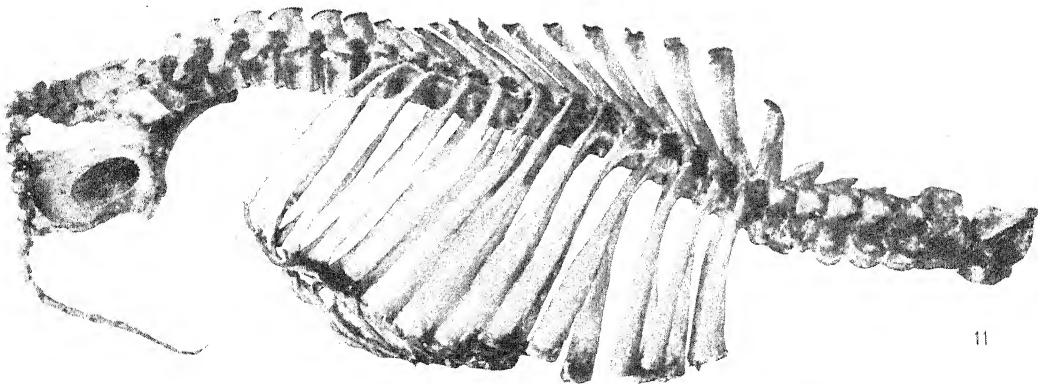
The application of the two methods is discussed. The opinion is expressed that the former of the two methods, both for practical reasons and because it is more general in scope, should be the main method, though the latter may be also useful in particular cases.

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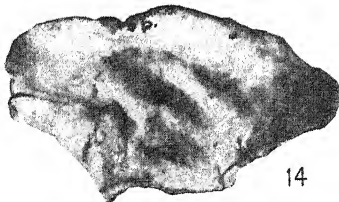
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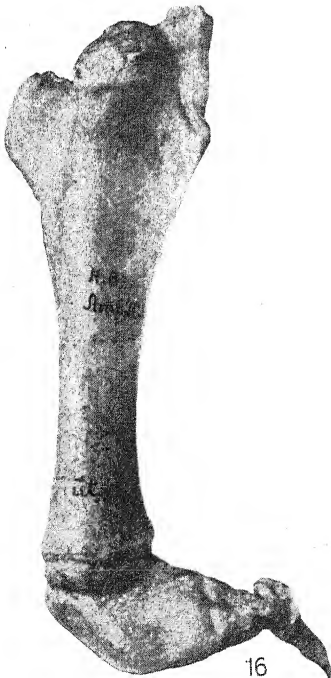
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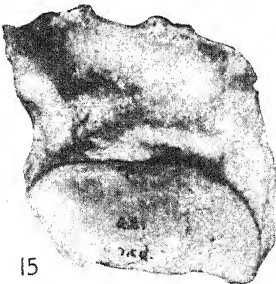
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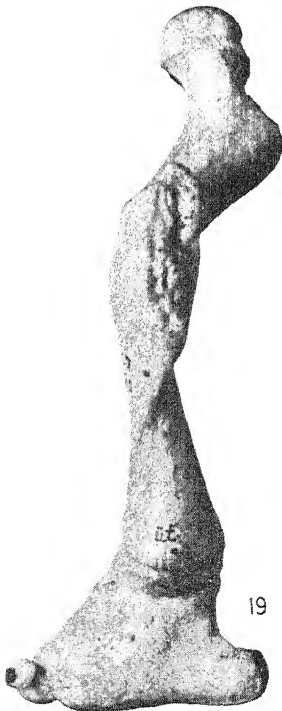
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EXPLANATION OF PLATES IX-XI.

PLATE IX.

- Fig. 1. New born short-spine calf.
- Fig. 2. New born amputated calf.
- Fig. 3. Skull of short-spine calf.
- Fig. 4. *a*, Deformed cranium of amputated calf. *b*, Mandibular rudiment from amputated calf with three incisors.

PLATE X.

(Skeleton of short-spine calf.)

- Fig. 5. Side view of deformed vertebral column.
- Fig. 6. Front view of deformed vertebral column.
- Fig. 7. Left costal bones.
- Fig. 8. Right costal bones.
- Fig. 9. Right os coxae.
- Fig. 10. Right scapula.

PLATE XI.

(Skeleton of amputated calf.)

- Fig. 11. Skeleton of trunk. NB. Deformed os coxae.
- Fig. 12. Sternum with xiphoid process and adjacent costal cartilages.
- Fig. 13. Hyoid bones.
- Fig. 14. Cartilaginous left scapula.
- Fig. 15. Right scapula, lower part ossified.
- Fig. 16. Rudiment of skeleton of right hind leg.
- Fig. 17. Rudiment of skeleton of right fore limb.
- Fig. 18. Rudiment of skeleton of left fore limb.
- Fig. 19. Rudiment of skeleton of left hind leg.

TYPES OF VISIBLE VARIATIONS INDUCED BY X-RAYS IN *DROSOPHILA*.

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(With Three Plates and Fifteen Text-figures.)

I. EFFECTS DUE TO ALTERATIONS IN INDIVIDUAL GENES.

THERE is no criterion whereby most of the mutations induced by X-rays can be distinguished from those of "spontaneous" origin. Like the spontaneous mutations, the great majority of the X-ray mutations are lethals, and it cannot be said that any higher proportion of the latter is lethal than of the former. This commonness of the lethal effect is only to be expected as a result of such random changes in genes, and does not destroy the usefulness of either natural or induced mutations in natural or artificial evolution, in view of the fact that non-lethal changes—some of which may be advantageous—are concomitantly produced, though with far less frequency.

The non-lethal "visible" mutations of course possess the greater intrinsic interest, even though the lethals, by reason of their frequency and the comparative scarcity of "doubtful" cases, afford the geneticist the best mutation index; and some of the induced visibles have therefore been chosen for illustration here. Many of them are in no way distinguishable from the spontaneous visibles, and some of those most commonly induced are clearly identical, not only in appearance but also genetically, with some of the best known spontaneous mutations. Instances in point are rudimentary wings and white eyes, both of which have arisen on numerous occasions after treatment. The same applies to miniature wings, forked bristles, star eyes, etc., which are among the mutations that have been most often produced.

On the other hand, just as new kinds of visible mutations are continually being found in untreated material (though only rarely, considering the vast number of flies looked at), so, too, among the induced mutations many are not exactly like any found earlier in the

Drosophila studies. Some of them resemble previously known mutations, but when tested genetically are found to lie at a different locus in the chromatin. An instance of this is "carnation" eyes (*car*), found by Patterson, which closely resembles the known mutants ruby, garnet, and pink eyes, but, unlike any of them, lies nearer the "right" end of the X-chromosome than any other known gene except bobbed (carnation being at the locus 65.5). Others present some difference from previously known mutants in the manner of their expression, yet are shown by genetic tests to lie at known loci, thus constituting, together with the previously known mutant or mutants of that locus, multiple allelomorphic series. A greater number of the induced visible gene mutations, however, are distinctive both in appearance and in locus.



Text-fig. 1. Snipped wings—drawing of wings of flies descended from the second induced snipped mutant.

Among the induced new allelomorphs of previously known mutants, special mention may be made of "snipped wing" (Text-fig. 1) and "dominant eyeless" (see Text-figs. 2 and 3 for its various expressions). The first of these (found twice in my irradiation experiments) is an allelomorph of the well-known recessive mutant, vestigial wings, but much more extreme than the latter in its expression, (1) because it is dominant, producing the phaenotypic effects shown when in heterozygous condition; (2) because when crossed to vestigial it gives a much more nearly wingless individual than seen in homozygous vestigial; (3) because, when it itself is homozygous, it acts as a lethal. The dominant eyeless shows a relation to the recessive eyeless similar in all these respects to the relation shown by snipped to vestigial, being dominant,

giving a more exaggerated type than the recessive when crossed to the latter, and being lethal when homozygous. These cases (like that of the

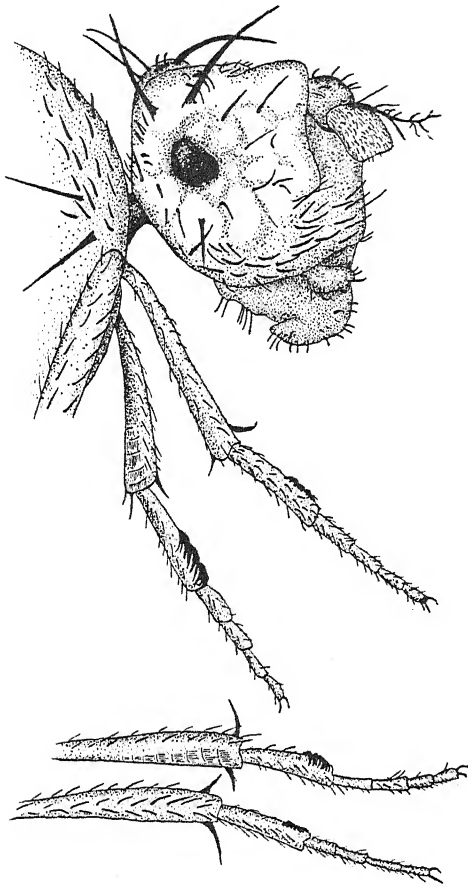


Fig. 2.

Text-fig 2. "Dominant eyeless" (heterozygous for normal), a more extreme allelomorph, lethal when homozygous, of the well-known recessive "eyeless" in the fourth chromosome. Note enlarged sex combs (in comparison with sex combs of normal type, shown below); this peculiarity is not found in the recessive eyeless.

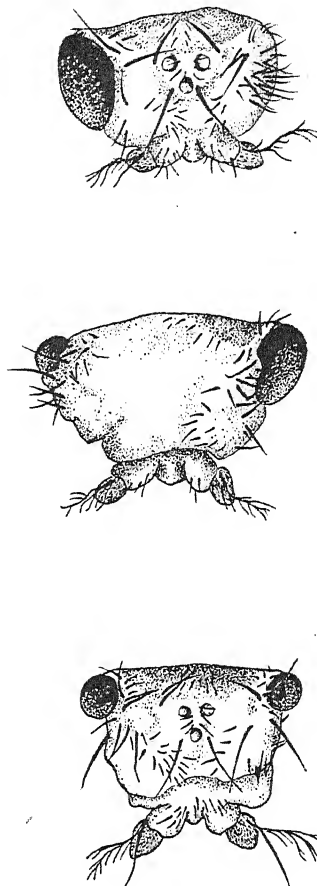


Fig. 3.

Text-fig. 3. Various appearances of "dominant eyeless" (heterozygous for normal), seen from above.

dominant lethal allelomorph of broad wings reported in untreated material by the writer (1928), and like truncate wings in comparison with its weaker allelomorphs) illustrate the fact that many lethals, even

when dominant, are merely "point mutations" belonging in the same general category as the recessive visible mutations, but more drastic in their effects on the organism¹. Among the "non-lethals," be it also noted, a graded series of forms may be picked out (sometimes at one and the same locus) showing different degrees of inviability, until the semi-lethal, and finally the fully lethal type is reached.

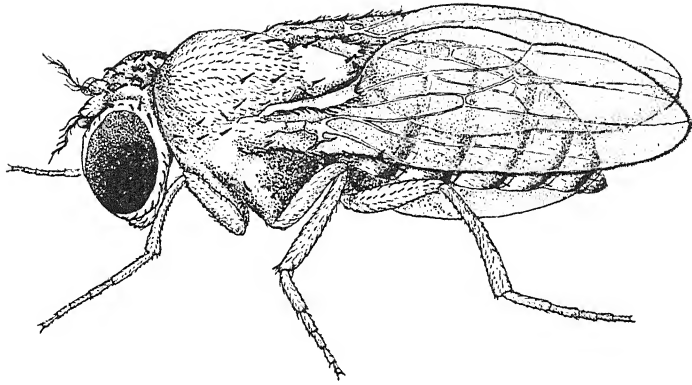
Other extreme (but non-lethal) allelomorphs that have arisen after X-raying are "scutex" (*sc^x*), a poorly viable allelomorph of the "scute" bristles condition, "echinex" (*ec^x*) which bears a similar relation to echinus, and "spectacled eyes" (*lz^s*) (Plate XII, fig. 1) found once by Patterson and once by Harris in rayed material, and twice by the writer as an apparently spontaneous mutation (it may have been found elsewhere also), where it behaved as an extreme allelomorph of the known "lozenge" eyes series.

The induced visibles are not always the most extreme possible allelomorphs known at their loci, even though that is often the case. Thus, although white eyes—the most extreme allelomorph of a long series known at its locus—has arisen in X-rayed germ cells over a dozen times, nevertheless eosin, apricot and other allelomorphs involving a more moderate degree of departure from the normal eye colour than white does, have also been induced at the locus of white eyes. Analogous cases are known at some other loci ("weakly forked" bristles, "cleftoid" wings, non-lethal "facet" eyes, etc.).

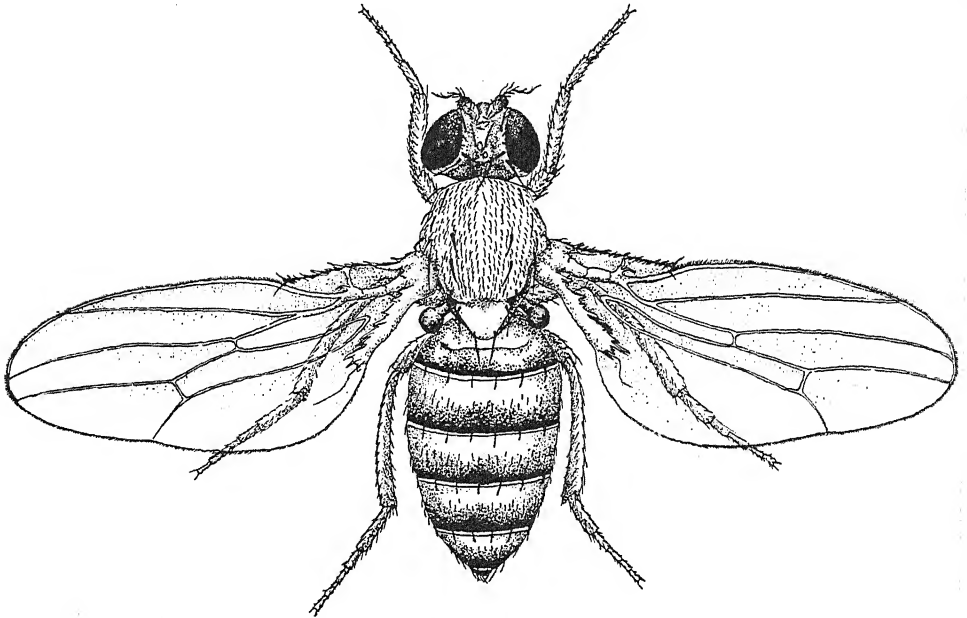
Text-figs. 4-7 illustrate a few of the induced visibles which do not seem to lie at the same locus as any previously known mutants. "Prickly bristles" (*Pr*), in Text-fig. 4, is a clear-cut dominant in the third chromosome, about 15 units from the "right" end. "Outstretched wings" (*o*), in Text-fig. 5, is a well-marked recessive located near the right end of the X-chromosome, a few units to the left of carnation. Bloated wings (*bl*), in Text-fig. 6, is a conspicuous sex-linked recessive, lying in an X-chromosome that had undergone a previous inversion.

At least as many of the induced visible mutations express themselves in an inconspicuous fashion, or to a variable extent, overlapping with

¹ The writer would therefore take issue with the assumption (Bridges, Mohr) that a lethal which, when crossed to a recessive visible lying in an apparently homologous locus, allows the latter to manifest itself (often in an exaggerated form) is, *per se*, probably a deficiency of an entire chromosome region surrounding this locus. In most cases it would seem more likely to be only a more extreme allelomorph produced by "point-mutation" at that specific locus, and probably should be regarded as such except where it can also be proved to give similar allelomorphic effects in crosses with one or more other mutant genes, lying at neighbouring loci.

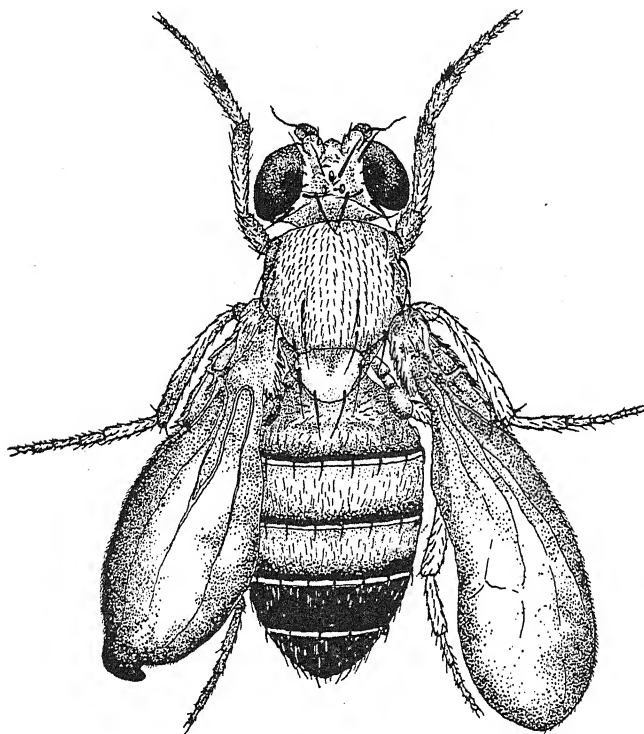


Text-fig. 4. Prickly bristles (heterozygous for normal)—a useful dominant (lethal when homozygous) located near rough eyes in the third chromosome.



Text-fig. 5. Outstretched wings—a new recessive of good classifiability at locus 62.5 in the X-chromosome ("standard" of 1925).

the normal type, as show definite and conspicuous character changes. These of course do not serve so well for illustration, or as genetic tools. One example has been illustrated in Text-fig. 7, the recessive sex-linked mutant "split veins" (*spl*). When this character is as pronounced as in the specimen used for the figure, there is of course no difficulty in detecting it, but its phaenotypic fluctuations are very great, as well as

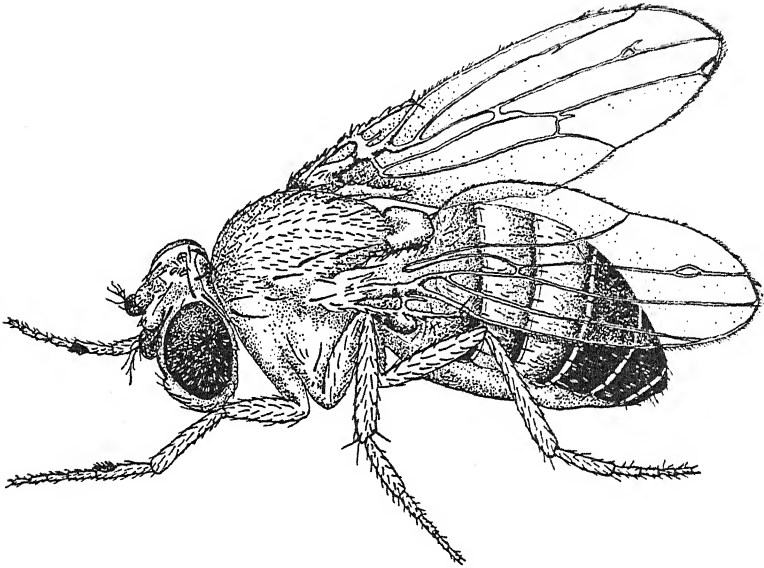


Text-fig. 6. Battered wings—a sex-linked recessive located in an X-chromosome containing a non-lethal inversion ("♂ 49") induced by irradiation.

manifold, and many specimens are indistinguishable from the normal. This is true also of many spontaneous mutations in *Drosophila melanogaster*, but in the past such cases, when not overlooked, have often been discarded as too troublesome to serve as practicable material for genetic use. This practice has had its justification, inasmuch as those samples of indefinite or variable mutations which were subjected to painstaking analysis—e.g. truncate wing, beaded wing, "with" pattern, dichæte bristles—always proved to depend upon stable genes, inherited according

to the orthodox Mendelian rules, the variability being due (aside from modifying factors) to fluctuations in the *expression* of the gene.

It may be emphasised, before leaving the subject of gene mutations produced by X-rays, that no evidence has been found which casts doubt upon the stability of the mutant genes that have arisen through the induction of simple "point mutations." Numbers of stocks of these mutants have now been carried along for over three years (some 75 generations) since their origination, without any evidence of reversion on the part of the gene in question.



Text-fig. 7. Split veins—a sex-linked recessive of variable expression, overlapping the normal type; the specimen illustrated is an extreme example.

II. EFFECTS DUE TO CHANGES IN THE "DOSAGE" OF GENES.

(a) *Heteroploids involving portions of a chromosome.*

The breakages of chromosomes brought about by X-rays, accompanied sometimes by re-attachment of the broken-off fragment or fragments to a different point in the chromosome from before, or to a different chromosome, give opportunity for the origination of visible variants due not to gene mutations but to gene disproportions ("unbalance")—*i.e.* to the presence of some chromatin parts, with their contained genes, in different numbers from the rest. For in generations

subsequent to the breakage it is possible for some individuals—"hyperploids"—to inherit the chromosome fragment (attached or unattached) in addition to two otherwise normal sets of chromosomes; these individuals have an excess of the genes of the kind present in the fragment, in comparison with a normal number (ordinarily two except, in males, for genes in the X and the Y) of the other genes in the cell. Other individuals—"hypoploids"—may fail to inherit the fragment, although inheriting the incomplete chromosome from which the fragment was broken off. These have a deficient number of genes of the kind in question, in comparison with a normal number of all the other kinds of genes. In cases of mutual translocation (segmental interchange) individuals may be formed that are hyperploid in regard to one segment of the chromatin, and at the same time hypoploid for another segment.

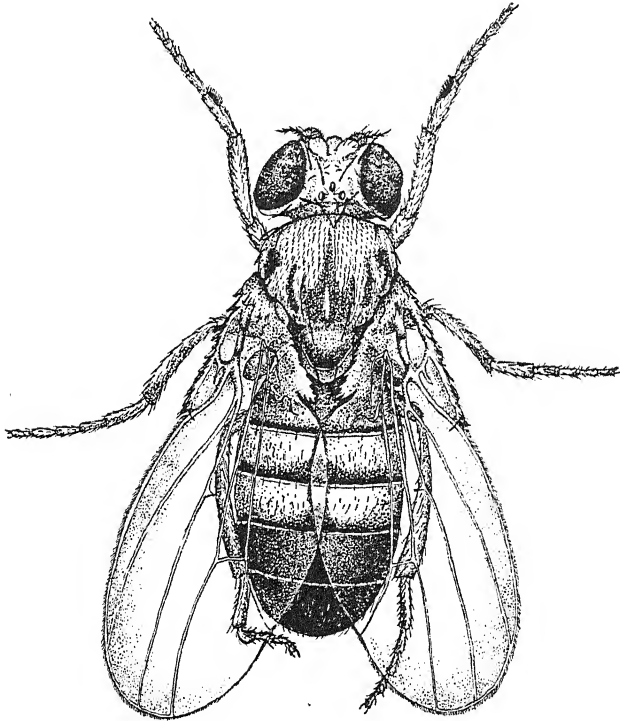
It is to be expected that many of the above kinds of individuals will be abnormal (often to the point of being lethal) even though none of their individual genes are abnormal, just as, in following out a recipe for a cake, we may produce an unpalatable result by adding too much or too little of certain constituents, *in proportion to the others*¹, quite as well as by adding a constituent of the wrong quality. That is, in fact, all there is to the so-called "theory of genic balance," whether as applied to sex or to anything else. The present work demonstrates the applicability of this "theory" (if we may thus dignify so obvious a proposition) in *Drosophila* to cases of excess or deficiency not involving entire chromosomes, but only parts, sometimes small parts, of chromosomes.

It should eventually be possible to discover, by observation of the phaenotypic consequences of the various chromosome abnormalities that can be induced by X-rays in *Drosophila*, the results produced by the addition or subtraction of genes in every portion of the chromosome complex. This is not possible when we are dealing with entire chromosomes, since excesses or deficiencies of great amounts of chromatin usually cause death; and even if they do not, the question must arise as to which parts of the chromosome are responsible for the observed effects. Thus, Bridges's work on offspring of triploids has shown that flies with an extra second chromosome, or with an extra third chromosome, but with all the other chromosomes in normal amount, always die before hatching. By breeding flies having induced excesses or de-

¹ "In proportion," of course, because if all the constituents are reduced or increased by the same factor, we get a cake of the original quality and merely of different size, or showing differences—*e.g.* overbaking—purely dependent on its size (volume-surface relations).

ficiencies of *parts* of the long autosomes, however, it should be possible to obtain some living heteroploid progeny, in which the phaenotypic abnormalities may be studied and compared with the effects of mutations at known loci in the regions involved.

The fly shown in Text-fig. 8 is a hyperploid of the sort referred to. It carries as excess a portion of the left end of the third chromosome

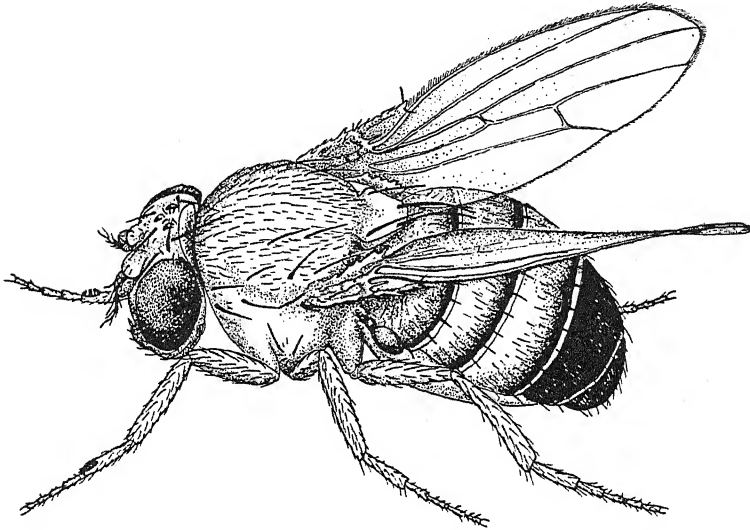


Text-fig. 8. Hyperploid containing an extra segment from the left end of the third chromosome (attached to the *Y*-chromosome).

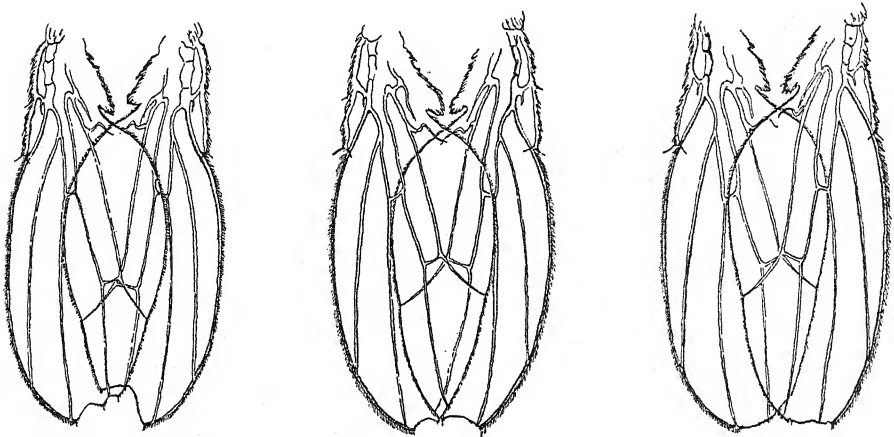
over 25 units long (including the loci of roughoid and hairy) attached to the *Y*-chromosome, and it carries also two normal third chromosomes¹. It has numerous abnormalities—broad, convex wings with imperfect cross veins, reduced bristles, dark-patterned thorax, misshapen eyes, a tendency to incurved hind legs, sterility. Text-fig. 9 shows a hyperploid due to an extra piece of the right end of the third chromosome, over 30 units long, attached to one of the second chromosomes¹, in addition

¹ Cytologically illustrated by Painter and Muller (1929).

to two normal third chromosomes. It has different abnormalities—divergent, slightly raised wings with imperfect fifth longitudinal veins,



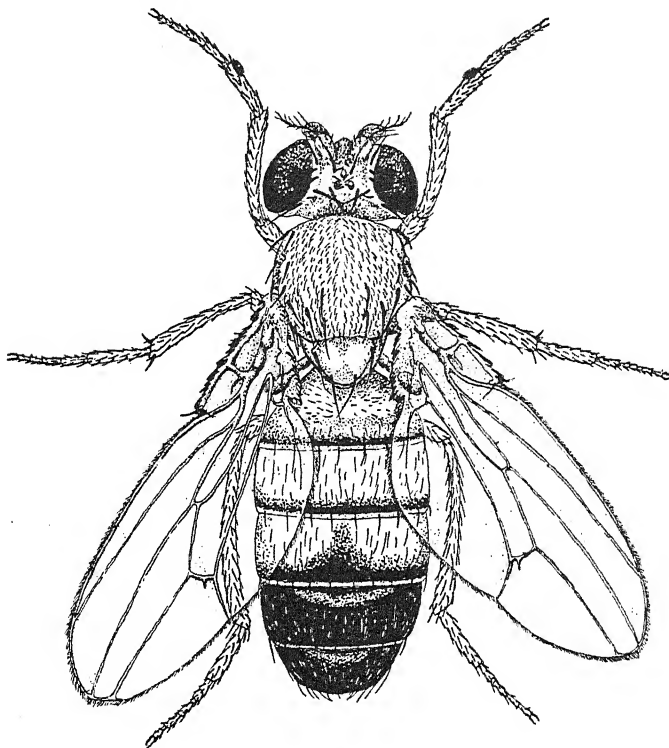
Text-fig. 9. Hyperploid containing an extra segment from the right end of the third chromosome (attached to the second chromosome).



Text-fig. 10. Wings of hyperploid containing a segment extracted from the left arm of the third chromosome (attached to the right end of an entire third chromosome).

long bristles, rather small eyes, sterility. In still another case of hyperploidy of a part of the third chromosome a fragment taken out of the

left arm of the chromosome, including the locus of hairy, but not extending to the end, has become attached to the right end of an unbroken third chromosome. This excess, probably in connection with an otherwise normal collection of genes, gives rise to flies with wings like those shown in Text-fig. 10; these flies seem otherwise normal and are fertile. (In

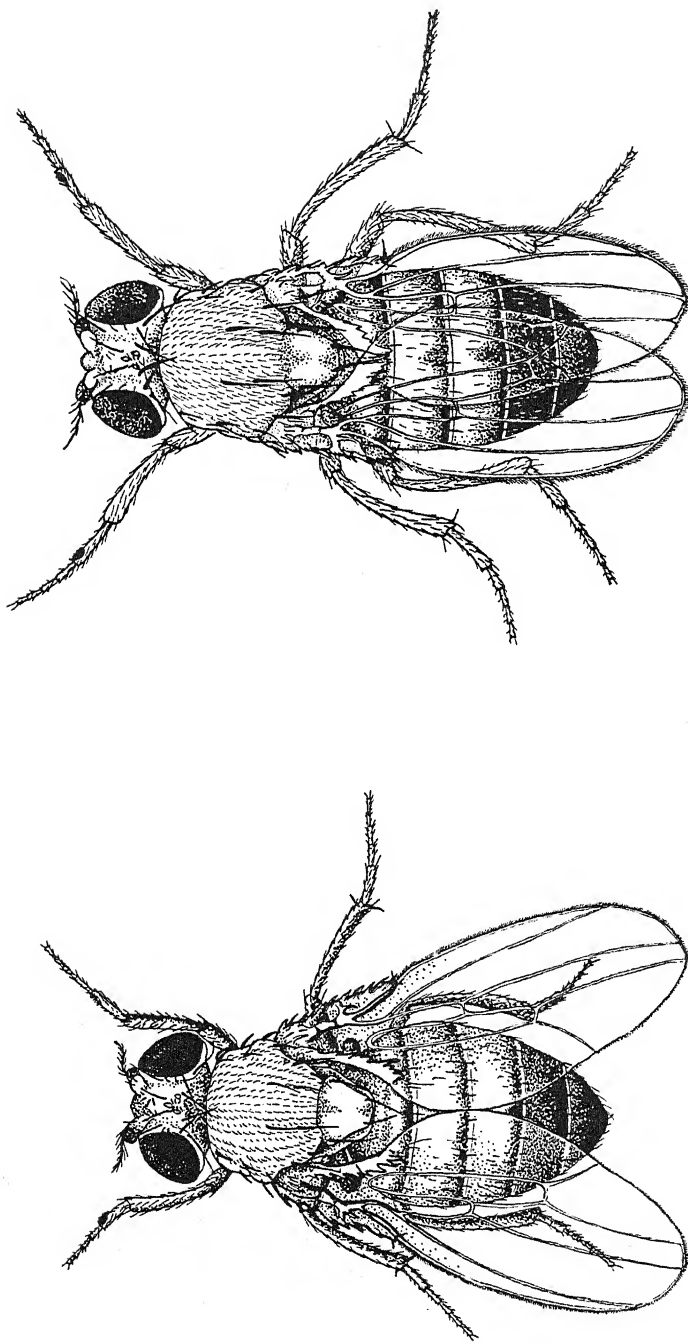


Text-fig. 11. Hyperploid male containing besides a normal X -chromosome, a deleted X consisting of a segment from the left end (broken off between the loci of prune and white) attached to a segment from the right end (broken off between the loci of carnation and bobbed).

this case there is a remote chance that a concomitant, linked gene mutation was responsible for the visible effect.)

Text-fig. 11 illustrates hyperploidy of parts of the X -chromosome. In males of the type shown there is, besides the normal X and Y , a "deleted" X about a third or fourth the length of a normal X ¹, composed of a section from each end of the chromosome, joined together, with the middle left out. Phaenotypically, this produces a tendency to

¹ Cytologically illustrated by Painter and Muller (1929).



Text-fig. 12. On left, hyperploid called "Tubby," containing, besides a normal set of chromosomes, a small free fragment of undetermined origin; on right, normal fly for comparison.

narrow, divergent wings with somewhat branched veins, coarse, often doubled or supernumerary bristles and hairs, and a rather blunt abdomen. Since such males are fertile, it is evident that we have narrowed down the locus of the specific sex differentiator (or, less probably, in view of this, the sex differentiators) to the portion of the X-chromosome missing from the deleted X.

In Text-fig. 12, the hyperploid called "Tubby" is illustrated, in contrast to a normal fly. Here only a small portion of a chromosome, existing as a free fragment with its own spindle fibre connection¹, is present in addition to the normal collection of chromosomes, but the dosage of the particular genes involved is evidently of consequence, since the effect of the overdose is quite conspicuous—producing a much shortened fly, with all its transverse dimensions increased in proportion to its longitudinal ones; the wings also tend to be divergent, and the eyes bulgy. The derivation of the chromosome fragment responsible for this has not yet been determined.

Although, on the whole, the effects of a large genic disproportion, when not lethal, are more diffuse, involving a greater number of characters slightly, than those of the average "point mutation," nevertheless in any particular case a genetic or cytological analysis is necessary to decide with which phenomenon one is dealing. In the case of "Tubby," for example, there was nothing to indicate that an ordinary dominant gene mutation was not involved, except the surprising fact that it was inherited as though in a fifth independent linkage group.

In addition to cases of genic disproportion brought about, as above, by the inheritance of translocated or free chromosome fragments, other cases have been encountered, obtained more indirectly as a result of irradiation. In these the immediate effect of the irradiation had been to produce some sort of inversion of a chromosome, as represented diagrammatically in Text-fig. 13*b*. In a later generation, this inverted chromosome may undergo crossing over ("single"), in some cases with a normal chromosome, in other cases with another inverted chromosome having a somewhat differently arranged inversion (see Text-fig. 13*c*). One of the crossover chromosomes resulting will then have one or more of its regions twice represented ("duplication"), while the other will entirely lack the genes of this region or regions. Thus hyperploids and hypoploids arise, in which the overdose or underdose of genes concerns some limited section located in some other than the terminal region of a chromosome. Cases of this sort, found in the X-chromosome, have

¹ Cytologically illustrated by Painter and Muller (1929).

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Text-fig. 13. Diagram illustrating the formation of deficient and of duplicational chromosomes by crossing-over between chromosomes having non-identical but overlapping inversions.

(Points of breakage or attachment indicated by dots placed above or below them.)

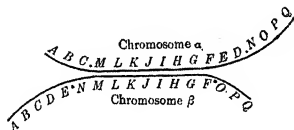
(a) Diagram of the original normal chromosomes, showing the points where they are to become broken and re-attached.

Chromosome α
Chromosome β
A B C . D E F G H I J K L M . N O P Q
A B C D E . F G H I J K L M N . O P Q

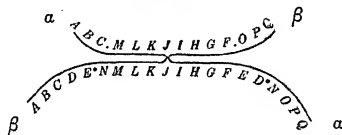
(b) The inversional chromosomes resulting after breakage and reversed attachment at points above shown.

Chromosome α
Chromosome β
A B C . M L K J I H G F E D . N O P Q
A B C D E . N M L K J I H G F . O P Q

(c) Synapsis in the heterozygous individual formed by crossing parents having chromosomes α and β , respectively.



(d) Crossing-over in the above heterozygote.



(e) The resulting deficient and duplicational cross-over chromosomes.

A B C . M L K J I H G F . O P Q (The deficient α - β chromosome.)
A B C D E . N M L K J I H G F E D . N O P Q (The duplicational β - α chromosome.)

Text-fig. 14. Diagram illustrating the formation of hypoploid and of hyperploid combinations by crossing of individuals having non-identical but overlapping translocations.

Translocational Parent a	Translocational Parent b
<u>A B</u> <u>C D E F G H I</u> <u>A B C D E F G H I</u>	<u>A B C D</u> <u>E F G H I</u> <u>A B C D E F G H I</u>
Hypoploid Progeny of a and b	Hyperploid Progeny of a and b
<u>A B A B C D E F G H I</u> <u>E F G H I</u>	<u>A B C D A B C D E F G H I</u> <u>C D E F G H I</u>

(Genes lettered only in chromosomes of the kind subject to breakage; points of attachment indicated by dots placed above or below them.)

further widened our knowledge of the phaenotypic effects of altering the dosage of genes in different parts of this chromosome, and have thereby narrowed down still further the locus of the specific sex-differentiating gene (or genes). Other cases of an essentially similar kind have resulted from two successive, partially overlapping inversions ("re-inversion"), followed by crossing over between the "reinverted" and a normal chromosome.

Another way in which it will be possible to produce zygotes having a disproportionate share (excess or deficiency) of some limited region, not at the end of a chromosome, is by the crossing of individuals both containing a translocation of the same general chromosome region, but one of which has a larger translocation than the other. As shown in Text-fig. 14, some offspring must thereby be formed which contain the abridged chromosome from one parent together with the attached section from the other parent. Offspring which have the attached piece derived from the parent with the longer translocation will then have an excess (triple amount) of genes of the sort present in the proximal portion of the translocated piece (the part in regard to which the two cases of translocation differ), while offspring of the complementary composition will have a deficiency (haploid amount) of the same non-terminal region. Crosses of this kind have not, as yet, resulted in viable heteroploid or hypoploid zygotes, owing no doubt to the regions in which the two crossed translocations differed being too long or too "potent"; but as practically all regions, and all lengths, however minute, of the chromatin may thus be tested through the use of different combinations of translocations, it should eventually be possible, by means of this method and of that outlined in the preceding paragraph, to determine the effects of genic disproportions of all portions of the germ plasm, and to compare these with the effects of gene mutations in the same regions.

In some of the cases of crossing over between chromosomes having an unlike gene arrangement, the hypoploids, as well as the hyperploids, have been found to be viable, whereas in the cases of translocation thus far dealt with only the hyperploids, or neither, have been viable. Inviability is the more to be expected, the larger the chromosome region concerned and the greater the departure from the normal "dosage." It is easily seen that subtracting a given amount from a normal chromosome complex produces a larger proportionate change in the dosage than does adding the same amount, for the change from two doses to one dose of any given gene is a subtraction of 50 per cent., whereas the change from two doses to three is an addition of only 33 per cent. Hence

the more drastic effect of the disproportion on the viability of the hypoploids as compared with that of the hyperploids is in general to be expected.

(b) *Inter- v. intra-chromosomal genic disproportion.*

The cases of hyperploidy in which parts of the *X*-chromosome have been involved have always agreed in showing the male to be affected to a more extreme degree than the female. Thus, the hyperploid males of the composition " $Y + X + (X -) + 2A$," where $(X -)$ represents some portion of the *X*-chromosome not containing the specific sex determiner and *A* represents the autosomes, in each case were less normal than the hyperploid females of the composition " $2X + (X -) + 2A$." This finding leads to a rather peculiar conclusion.

So far as the relation of *X*-chromosomal genes to autosomal genes is concerned, the hyperploid females in question represent a greater departure from a condition normal to the species than do the corresponding hyperploid males. For in such females the genes of the kind present in the extra portion exist in triplicate, the number of each of them bearing to the number of each kind of autosomal genes the ratio 3 : 2. This is the same ratio that holds in "superfemales" ($3X : 2A$), except that in the latter it applies to all genes in the entire *X*-chromosome, including the sex differentiators. Since in these superfemales this genic disproportion results in considerable phaenotypic abnormalities and (ordinarily) a very low viability, it might be expected that these same effects would be repeated, though probably not in their entirety, when a large portion of the *X* (even one not including the sex differentiators) was present in these proportions. In the corresponding hyperploid males, on the other hand, the genes present in the "extra" portions of the *X* bear the relation 2 : 2 to the autosomal genes, just as they do in normal females. The latter ratio then could not in itself lead to any phaenotypic abnormality of these flies, except conceivably to some superposition of female-like characteristics upon an otherwise male organism.

Actually, the abnormalities of the hyperploid females in question have usually been found to be rather different from those in triple-*X* "superfemales," and in the main very similar, though in lesser degree, to those found in the corresponding hyperploid males (when the latter are viable enough to exist). And these males, in turn, though in some cases sterile, do not seem to be partially female in character, but exhibit other more distinctive peculiarities. Hence these abnormalities cannot be due to the *X* : *A* disproportionality. The only possible

remaining explanation¹ of them lies in the disproportionality which exists in such individuals between genes in different regions of the *X*-chromosome itself. Those of the kind present in the extra portion of the *X* bear to those in the remainder of the *X* the ratio 3 : 2 in the hyperploid females and 2 : 1 in the hyperploid males, whereas in all normal males and females the relation of these genes is 1 : 1. Since this disproportion involves the same genes, and is in the same direction in both sexes, although greater in the males, it is only to be expected that, if the phaenotypic abnormalities were due to this cause, they would be similar in the two sexes, but more extreme in the males, just as has been found.

Hence the peculiarities in question are due to what may be termed "intra-chromosomal genic disproportion" (or "unbalance"), in distinction to "inter-chromosomal genic disproportion." It is the latter disproportion which causes the somatic abnormalities whenever entire chromosomes are involved, and which, as would be expected, has been found to be the prevailing cause of them even in most previously known cases of heteroploidy of chromosome portions (*e.g.* in the "secondary mutants" of *Datura*). The expectation that this would be true is based upon the fact that, when a portion of a chromosome is missing or redundant, there is a much greater number of genes ordinarily involved in the resulting inter-chromosomal disproportion than in the co-existing intra-chromosomal one. This being true, we may ask, why should the effects of the intra-chromosomal disproportion be relatively so much more pronounced than those of the inter-chromosomal disproportion in the present series of cases?

It is possible that only a few genes are concerned in producing most of the observed effects here, and that these just happen to lie in the same (the *X*) chromosome. If, however, the phenomenon represents a general tendency for the *X*, a plausible explanation is to be found for it in the past history of the species and of its ancestors. Somehow the species has already become adjusted to having either the $1X:2A$ or the $2X:2A$ relation (for males and females, respectively) without the individuals of either sex showing "abnormalities." Apparently, too, the main sex differences are not thus determined, except by one or a few specific genes in the *X* (the "sex differentiators"), the proportion of which to certain of the *A* genes is decisive. Since the organism is already adjusted to withstand with perfect impunity a 100 per cent.

¹ Since the addition of different deletions results in much the same effects, regardless of exactly where the breakage occurred, these are not "position effects" caused by displacement of certain genes from others previously adjacent to them.

change of just this kind (*i.e.* in the proportion of all the non-sex differentiating genes in the *X* to those in the autosomes), a similar but somewhat greater change in these approved proportions (of *X* to *A*), resulting in a ratio $3X : 2A$, would be unlikely to disturb greatly the normal processes of morphogenesis and physiology. On the other hand, the genes within the *X* have for a long time been constant in their ratios to one another, intra-chromosomally, so that there has been no opportunity for the species to become adapted to tolerate changes in these intra-chromosomal ratios, without abnormal manifestations resulting. Hence such a change in the *X*'s (hyperploidy of a *part* of an *X*) as produces a simultaneous alteration of the inter- and intra-chromosomal ratios is likely to cause more effect through the latter disproportions than through the former.

The heteroploids involving portions of the *X* that have been dealt with in the present work have been as follows:

(1) Several translocations in which the *X*-chromosome has been broken at a point near the "left" end of the ordinary genetic map (to the right of the locus of white eye). Actually the chromosome in these cases is broken somewhat near its middle, since the left end of the map is ordinarily shown on a much smaller scale than the rest of the chromosome (see discussions by Muller and Painter, 1929). In these cases the hyperploids having a redundancy of the left-hand region show a considerably higher degree of morphological abnormality, and also of inviability, in the male than in the female, while the hyperploids having a redundancy of the longer right-hand fragment, including the sex differentiators, are inviable, no matter whether they contain, in addition to the fragment, one normal *X* or two. Such inviability must clearly be due to intra- and not to inter-chromosomal disproportion. Translocations involving breaks to the right of the sex differentiator have not yet been found and analysed.

There have, however, been (2) numerous "deletions" in which the middle of the *X*, including the sex differentiator(s), has been lost, and the left end (sometimes of considerable size, including the loci of white and facet; sometimes smaller) has become joined to a piece of the right end. In some of these cases the male hyperploid is completely inviable; in others, in which the redundancy is smaller, the male is viable but more abnormal than the corresponding hyperploid female. Only a few¹, however, have been analysed at all fully, and these have not yet included any cases in which a large piece of the right end and a small piece of the left end were involved.

¹ *I.e.* about 8.

(3) Heteroploids formed by crossing over (at homologous loci) between two chromosomes having the genes somewhat differently arranged, as shown in Text-fig. 13. These still require considerably more "mapping." In a number of cases the females bearing one such abnormal chromosome have been viable, though sometimes abnormal and sterile; in other cases both opposite types of single cross-over females are viable. The corresponding males have been found viable in only one case. The complete inviability of the heteroploid cross-over males in the other cases of this kind thus far studied may, however, be due only to their containing deficiencies of certain regions.

It will thus be seen that the previously found rule of the more drastic effect of intra-chromosomal than of inter-chromosomal genic disproportion in the case of the *X*-chromosome cannot yet be generalised to apply to all parts of that chromosome, but it certainly holds in most cases in which breaks in the region to the left of the sex differentiator are considered¹.

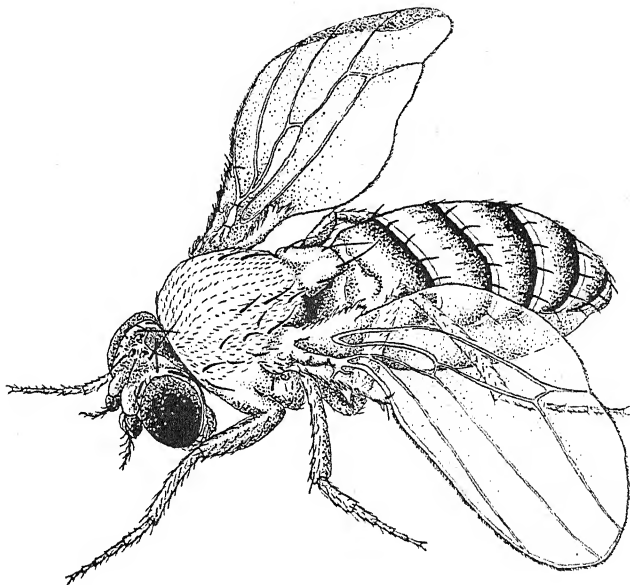
¹ In a paper by Muller and Dippel (1926) certain cases, that had been found by the junior author, were reported, in which females of a given class were absent but males were present. To explain these cases it was assumed that the double *X*-chromosome that had been present in the mother had been broken, so as to form a *J* in the shorter arm of which (consisting of the "right" end of an *X*) the sex-differentiator was absent. It was inferred that such a *J*, in combination with a *Y* and with normal autosomes, would result in a viable male, but, in combination with a single entire *X* and with normal autosomes, in an inviable female. This difference was based on the greater inter-chromosomal disproportion which would have existed in the case of such a hyperploid female than in that of the male, for the superior importance of intra-chromosomal disproportion in breakages involving the *X* had not yet been discovered. It will be seen that the cases described in the text give rise to an expectation contrary to that assumed in the paper here referred to. If that inference was incorrect, then various conclusions following from it were also incorrect, *e.g.* the existence of a "genetic sex reversal," and of embryonic predetermination of the sex of the future germ-cells. Hence the true explanation of the results is not clear, and might have to be sought in some peculiar fault of the technique. Unfortunately, we were no longer in a position to follow up the cases experimentally or cytologically, after the unusualness of the results had become apparent.

As yet, however, it would not be legitimate to generalise from the cases given in the text to the cases here referred to, because in none of the former is the excess portion of the *X*-chromosome derived from the right end alone, nor is it of sufficient size to test the matter. The ratios borne by certain particular genes from the left-hand region of the *X* to those in the middle may, as above mentioned, have been responsible for most of the effects of intra-chromosomal disproportion hitherto observed. In that case, a redundancy of a different part of the *X* would not be expected to follow similar rules in its expression. This would be a pertinent point if, as is quite possible, the different portions of the *X* have had a different kind of evolutionary history, such as having been translocated from autosomes at some period in the past.

III. "EVERSPORTING DISPLACEMENTS."

(a) *Description of cases.*

A rather surprising finding, in connection with translocations and other changes in the arrangement of the genes, was the fact that, even when all parts of the chromatin appeared to be represented in the right dosage—though abnormally arranged—the phaenotypic result was not always normal. In fact, both translocations and inversions more often



Text-fig. 15. "Swooping" wings—illustrating the dominant phaenotypic effect accompanying one of the induced translocations between the second and third chromosomes; lethal when homozygous. This translocation was of the "mutual" kind—both second and third chromosomes having become broken and having interchanged segments.

than not seem to have a recessive lethal action associated with them; *i.e.* flies in which the same gene re-arrangement has been received from both parents (or, in the case of the *X*-chromosome of the male, from one parent) die before maturing, in the case of more than half of the re-arrangements. Moreover, those re-arrangements which are not accompanied by a lethal effect commonly produce sterility and morphological abnormalities when homozygous. The reason for these effects has not yet been worked out satisfactorily, it being unknown whether they are due to the morphogenetic effect of the re-arrangement in itself, or to

some gene mutation or gene loss ordinarily accompanying the re-arrangement. Apparently the gene re-arrangements in *Oenothera* (see Darlington, 1929) are not so often lethal.

Where there are many recessives we should expect a few dominants. A number of dominant character changes accompanying translocations and other gene re-arrangements have, in fact, been found. One, called "Swooping," is shown in Text-fig. 15. (The wings are sometimes carried in the position shown, at other times they are placed normally, but they are always arched.) In this variant there has been a translocation between the second chromosome and the third. Another translocation was found to be accompanied by a dominant minute-bristle effect, a third by dominant thoracic malformations, a fourth by a newly arisen "star" eye. Other, more peculiar, types will be referred to presently.

The above-mentioned variants, involving chromosome abnormalities, have no known visible features whereby they may be distinguished as a class from ordinary gene mutations. But, in addition to these, a group of distinctly peculiar variants accompanying chromosome abnormalities has been found. These I am provisionally terming "eversporting displacements¹."

The finding of the first case began with the discovery in 1927 of a dominant "Notch" wing, sex-linked and lethal to the male, appearing in the daughter of an X-rayed male. Some dozens of Notches have been found before in the history of the *Drosophila* work, in untreated material, and a few of them have been of special interest. These were the so-called "Notch deficiencies" (Mohr, 1919), which contained, besides the "mutation" to Notch, a mutation to a white eye allelomorph in a nearby locus, and probably other mutations or losses in the intervening and adjoining loci. In order to test out whether the new X-ray Notch was such a deficiency, it was crossed to a stock containing white eye (and yellow body). This cross would give any recessive mutation to white, that might be accompanying the dominant Notch, a chance to manifest itself in the offspring. To the great surprise of the writer, however, the Notch-winged offspring of this cross had neither white nor normal red eyes, nor even eyes of any uniform intermediate colour. They had mottled eyes, and exhibited various grades and sizes of lighter and darker areas,

¹ The term "displacement" is here used to designate any kind of change in gene arrangement, involving the breakage of a chromosome and the re-attachment of one or more of the pieces at a different point than before. "Displacements" thus include translocations, deletions, inversions, and duplications—to mention only hitherto analysed kinds of changes in gene alignment.

ranging in tinge through and between the whole series of known allelomorphs of white eye. A mottled fly of this race, of predominantly light hue, is shown in Plate XIII, fig. 1. If this Notch were due to a deficiency, the deficiency was certainly not complete at the locus of white eye. Hence there seemed little reason for assuming a deficiency in this case.

Since the developmental influences that operate in the formation of the compound eye seem ordinarily to act in a very regular and uniform manner on the different elements or ommatidia, and since results from gynandromorphs and from fractional or somatic mutations indicate that these ommatidia are largely self-differentiating, so far as pigment formation is concerned, it was regarded as probable that the mottling of the eyes here encountered was due to a genetic diversity of the different eye-forming cells. This, if true, meant that the chromosome or gene controlling the eye colour in this case must be subject to frequent genetic changes during eye development, *i.e.* must somehow be "ever-reporting," somewhat like the genes for variegated pigmentation in corn and in some other plants. In that event, it might be that similar genetic variations were occurring in the germ tract also. This would result in offspring showing different grades of mottling, or perhaps even full red or white, that tended in turn to transmit their grade of coloration to their descendants (though again perhaps with variations plus and minus). Crosses were therefore made to test this possibility, and it was found that at least two distinct genetic strains could immediately be obtained from one original mottled line—a lighter and a darker. Typical specimens of each of these two strains are shown on Plate XIII, though there is considerable fluctuation apparent in each strain.

The darker strain has predominantly red eyes with scattered spots, usually small, that contain little or no pigment. These less pigmented spots appear as dark rather than light spots in the picture because the dark pigment lying at the bases of the neighbouring ommatidia is seen through their relatively transparent side walls when they are not exactly in vertical view—see paper by Casteel (1929), in which this has been proved by sections. In the lighter strain, on the contrary, the "ground colour" is usually very light, with a variable number of speckles of darker hue, often grouped towards the posterior border of the eye, and sometimes having in addition larger, apparently deeper and more diffuse areas, of a colour only slightly darker than the remainder of the eye.

The dark-eyed individuals of some strains, when bred to white males, yield both the dark and light types in comparable frequencies; in other

strains they yield almost exclusively darks like themselves. But the light individuals, no matter what their origin, have so far yielded, with rare exceptions, only the light type of mottled. The preliminary genetic tests indicate that both types, when derived from a dark parent, may have received the same assortment of chromosomes, at least so far as the orthodox linkage groups are concerned. Prolonged selection of both dark and light types, in the "plus" and "minus" directions respectively, has failed to produce a uniform normal or a uniform white type. In this respect the results differ from those obtained by the workers on variation in plants and by Demerec on his three frequently "mutating" genes in *Drosophila virilis*. They fail, moreover, to fit in with the idea of a gene composed of "gene elements" or "genomeres" (red- and white-producing, respectively) that are being separated at the time of chromosome splitting so as finally, after many divisions, to result in some genes containing all red and others containing all white elements.

In another respect, too, the results are different from what would be expected on the "gene element" or "genomere" theory of such phenomena. The notching of the wings, which is due to a gene at a locus $1\frac{1}{2}$ units removed from the gene for mottled eyes, is irregular in its manifestation in a given individual; it shows also considerable variation from one individual to another. Now these wing variations are not all phaenotypic, for it has been found that among the offspring of a given dark mottled individual that yields both lights and darks, the lights consistently average much more extreme Notch than the darks, which often show no Notch at all (cf. Plate XIII, figs. 1, 2); the light progeny so produced tend to transmit their more extreme Notch to their own light mottled offspring, whereas the dark progeny tend to segregate again into two major classes (dark, less Notch and light, more Notch) in the following generation. This makes the Notch character "eversporting" in the same way, and in parallel with the mottled character, and indicates that the phenomenon does not depend upon an assortment of individual "gene elements," or of any particles within and smaller than a single gene, but rather depends somehow upon the peculiar behaviour of chromosomes or segments of chromosomes, larger than and containing more than one gene.

Further evidence in favour of a chromosome abnormality, rather than an unusual intragenic situation, was adduced when it was found by genetic tests that the X-chromosome containing mottled is broken, and that the "left-hand" fragment, containing both the mottled and

the Notch loci, is attached by its broken end to the third chromosome. The break was at some distance to the right of Notch, and there are some unmutated genes in the right portion of the fragment, between Notch and the break, and others in the left portion between the left end of the chromosome and mottled. Crossing over between the attached fragment and the homologous portion of an *X*-chromosome of normal structure sometimes occurs in the space between mottled and the left end, so that it has been found possible to get the gene for yellow body into the fragment with mottled. It is this fact which gives the information that the fragment must be attached at the end at which the breakage occurred.

There are other peculiarities, not yet unravelled, in the breeding behaviour of mottled. Thus, from one of the light lines, another rather dark line has been obtained, in which the males carrying mottled are able to live, though they are sterile. This line shows a greater range of colour variations than do the others, and shows very little notching except in the very light individuals, which are all females. From some lines, moreover, the hyperploids, inheriting the translocated fragment in addition to two ordinary *X*-chromosomes, are viable and appear in abundance; whereas, in others (including the dark line derived from the light line) no apparent hyperploids are to be found. All these peculiarities, although perplexing, nevertheless indicate that chromosome regions, affecting various different characters at once, are somehow concerned, rather than individual genes or supposititious "gene-elements."

It might have been suspected that the existence of a gene re-arrangement (translocation) in the mottled case was a mere coincidence, having nothing to do with the mottling of the eye. Recently, however, four more mottled allelomorphs of white have been found in experiments of the writer; examination of these should bring strong evidence as to whether the phenomenon of gene re-arrangement bears a causal or a casual relation to the mottling. All four of these mottleds resulted from the irradiation of mature sperm carrying the normal allelomorph, "*W*"; the mottleds which arose, were, like the first mottled, all recessive to normal but partially dominant to white. Notch wing was not involved in any of these latter cases.

One of these new mottleds—called "mottled 2" (*w^{m2}*)—was found by Dr Patterson in some material belonging to an experiment of the writer which Dr Patterson had kindly offered to examine. This mottled also breaks up into dark and light lines, the latter breeding true to light (not white) and the former segregating again. Some apparently uniform

reds are also produced by the darks. The mottled males are viable, but sterile. Crosses with *Dichaete* (in chromosome III) show linkage between the latter and the mottled locus; hence in this case there must be a translocation between the *X* and the third chromosomes.

Another of the new mottleds—"mottled 3" (w^{m3})—was found to be contained in a "deleted" *X*-chromosome—consisting of a fragment of the left end of the *X*, including mottled and extending beyond the locus of *facet* (which was not mutated), attached to a small fragment from the right end of the *X*, with all the intervening region omitted. Hence this case also involved a change in gene alignment. The mottling in this case has so far been seen only in males, when these carry, besides the deleted *X*, an entire *X* bearing the gene for white eye. Such males are sterile. The females of this race carry, in addition to the deleted *X*, attached *X*'s bearing the normal allelomorph of white and the gene for yellow body.

Mottleds 4 and 5 were found in the same experiment, and may have had the same parent, but they are almost certainly of independent origin, since they are genetically different. In both cases, the mottled males are viable and fertile, so that homozygous females can be obtained. These females are on the average considerably darker than the males, indicating that the variations in the two *X*'s of the female cells occur to some extent independently of one another, the darker showing greater dominance. There is no clear evidence as yet of a light mottled segregate from these races; the occurrence must be rather rare if it takes place at all. The breeding behaviour of both mottleds show peculiarities, although no detailed analyses have as yet been made. Mottled 4 was found to yield only five cross-over flies, including two double cross-overs, between *scute* and *forked* (the greater part of the length of the *X*-chromosome), in a total of 238 flies, though about 110 cross-overs, the great majority "singles," would occur normally in a count of this size. The usual conclusion would be that there has been a change in gene alignment, either a translocation or an inversion, in such a case. Mottled 5 gives much more abundant crossing-over than mottled 4; on the other hand, it yields flies which show all the morphological characteristics of hyperploids that carry as excess a portion of the left-hand region of the *X*-chromosome. This mottled, then, almost certainly involves a translocation.

It seems clear, from this list of cases, that there is some causal connection between the type of genetic instability in the somatic cells, involving the gene at the locus of white, which gives mottled eyes, and

the occurrence of a re-arrangement in the linear order of the genes¹. We may now examine some apparently related results, involving other loci.

It has been a curious and unexplained feature of all the *Drosophila* work to date (though apparently not hitherto realised) that although eye-colour changes form a considerable fraction of the known visible mutations (in non-rayed material), and although a good many dominant mutations of varied kinds have been found in all this time, nevertheless there have been practically no dominant mutations in eye-colour (at least not such as changed the normal red eye to a different colour). By contrast, the number of dominant wing mutations and bristle mutations (in proportion to the number of recessives having such expression) is striking. So far as the writer is aware, the first eye-colour change in *Drosophila* that was dominant to the normal was reported by Weinstein (1928) in the progeny of irradiated males. Weinstein also reported that this change was "eversporting," and that it involved a translocation, though the details have not yet been presented. Since then five dominant eye-colour changes have been found in our laboratory, all in the progeny of irradiated males².

The first of these, "Plum," found by the writer, is of yellowish colour on first emergence, but gradually changes to a bluish purple (not a reddish purple like that of "purple" eye). It is depicted in Plate XIV c, and the normal eye-colour is given at a, for comparison. Unlike any of the recessive eye-colours known, except the mottled allelomorphs of white just described, the pigmentation is not uniform throughout the eye, but shows darker and lighter patches of variable size, seen best in the older flies, but also noticeable in young flies in the deeper layers of the eye when the upper layers are shaded. Plum lies in the right half of the second chromosome. It acts as a lethal when homozygous. Just where it is located cannot be determined because crossing over in the right half of the chromosome is suppressed. There is some crossing-over in the middle region of the chromosome; the cross-overs in the left region are rare and are all double cross-overs. Thus there has been at least one, and probably two inversions in the origination of this chromosome.

Another case, "Discoloured" eyes (*Di*), was found by Harris in some

¹ Several more mottled allelomorphs of white have recently been found by Patterson, who has kindly given the present writer the privilege of stating that the results of their analysis thus far are in harmony with the foregoing.

² Another case, apparently of the same general type, has recently been found by Hanson and Winkleman (1929).

flies which carried the sex-linked gene for vermilion eye. Some of these flies presented the appearance shown in Plate XIV *f*, and were very similar to light mottleds. Others were much like vermilion, but showed light areas, especially evident in the lower levels of the eye on shading them from above. The discolorations of these darker flies were of very variable size, position, and intensity. Further study of the case, carried out by the present writer, showed that, as in the case of mottleds 1 and 2, the light individuals gave rise exclusively or almost exclusively to light Discoloured eyes, whereas the dark produced both dark and light in comparable numbers. The gene, however, proved to be located not in the *X* but in the second chromosome. When the gene for vermilion was removed from the stock, the Discoloured eyes appeared as in Plate XIV *g*, *h*, some (as in *g*) looking much like Plum, and, like it, showing irregularities in the distribution of pigment, and others (as in *h*) looking more nearly normal in colour but still showing pigment irregularities, somewhat resembling those of dark mottled flies (cf. Plate XIII, figs. 1, 2). Discoloured, like Plum, lies in the right half of the second chromosome, is lethal when homozygous, and *involves an inversion* in the right half (though not in the left half) of the chromosome in which it lies. When crossed with Plum, it gives a few flies carrying Plum in one second chromosome and Discoloured in the other. Their eyes resemble those of heterozygous Plum, but abnormalities are evident in other parts of the body. It is not clear, therefore, whether Plum and Discoloured should be regarded as allelomorphic.

When Plum was crossed with vermilion, the vermilion Plum combinations that were formed proved to have a coloration similar to that of the vermilion Discoloured, showing the irregularities of pigmentation much more distinctly than did the non-vermilion bearing the autosomal gene in question. These vermilion Plums are all much darker than the light segregates of the vermilion Discoloured, but lighter than most of the dark segregates of the latter.

"Moiré" eye (*Mo*) (Plate XIV *d*), found by the writer, leaves the fundamental colour of the eye unchanged, but causes marked irregularities in the dark pigmentation of the deeper layers. In eyes carrying also Plum it produces no distinguishable effect. It is located in the (homologue of the) left arm of the third chromosome, *in a region which has undergone an inversion*, since cross-overs between the left arm of a normal third chromosome and the homologous region bearing Moiré fail to occur, or, at any rate, to be viable. It is, however, not certain whether this case is analogous to the others, since the phenotypic abnormality

may be due to structural changes in the ommatidia that do not arise from genetic differences between the somatic cells.

"Tarnished" eye (*Ta*) (Plate XIV *e*), recently found by the writer, resembles Discoloured, being sometimes like the Discoloured of Plate XIV *g* and sometimes like Plate XIV *h*, or else of an intermediate grade. It was at first thought to be sex-linked, since it showed apparently complete linkage with sex-linked genes in the right end of the *X*-chromosome. At the same time this *X* produced a recessive lethal effect, located in this region, and showed throughout a much reduced frequency of crossing-over with normal *X*'s. Crosses with white eye proved that it was not an allelomorph of the white-mottled series, while crosses with autosomal genes showed it to be linked with both the second and the third chromosomes as well as with the *X*. Hence this case involves a double translocation, and the real locus of the effect is not as yet determined.

"Punch coloured" eye (*Pu*) (Plate XIV *b*), found by Oliver, seems different from the other dominant eye-colours in that it involves no obvious irregularity in pigment distribution. It may be recalled, however, that the irregularity of the type due to Moiré was not evident when Plum was simultaneously present; hence a negative result on this point is scarcely decisive. Punch was found by Oliver to be lethal when homozygous, but to be viable in heterozygotes which receive Plum from their other parent. He found it to be linked with genes of both the second and the third linkage groups, and hence to involve a displacement—namely, a translocation between the second and third chromosomes.

(*b*) *General discussion.*

Such are the main genetic facts to date concerning the "ever-sporting" conditions, and related phenomena, which have been induced by X-rays. Evidence is at hand that the somatic variegation is not confined to the eye. It has long been known that the membrane covering the testes contains a yellowish pigment. This must be in some way related to the red and the yellowish pigments of the eyes, since in flies manifesting the gene for white eyes, or one of the light allelomorphs of white, the testicular covering is colourless, or nearly so. Mottled males of various races have therefore been dissected, and their testes have been examined¹. It has been found, in each case, that the testicular

¹ The author wishes here to acknowledge with thanks the help of Dr T. Dobzhansky in the carrying out of some of these dissections and examinations.

membrane partakes of the mottled appearance. Instead of being coloured uniformly throughout, like the testes of normal individuals, the testes of the mottleds exhibit a patchwork of yellowish colour interspersed with colourless regions. The patches are large and clear-cut, the lines of demarcation being sharp.

Nor is variegation found only in characters related to the optic pigment. For example, in the writer's X-ray mutation experiment of 1926, a case was found called "mosaic bristles." In this the X-chromosome carried a recessive lethal, and had undergone an inversion or translocation that caused a suppression of crossing-over. This was accompanied by a dominant "roughening" effect irregularly distributed over the eyes, and by a modification of a few of the bristles. The latter showed the characteristic "forked" in a markedly sporadic fashion, one or more single bristles or small patches of bristles being forked anywhere on the surface of an otherwise normal bristled body, when the flies had also received the recessive gene for forked in the X-chromosome from the non-mosaic parent.

The eye colour is, however, the character *par excellence* adapted for a study of genetic variation in somatic development, because of (1) the regularity and uniformity of parts which it normally exhibits; (2) the fact that their arrangement is usually closely related to their cell lineage; (3) the multiplicity of these parts, which are nevertheless (4) capable of having the colour of all reviewed at practically one glance; and (5) the high degree of "self-differentiation" of the parts, at least in respect to colour. These are some of the reasons, no doubt, why the phenomena of "eversportingness," above described, have been noticed primarily in eye-colour. They do not, however, explain why most of the *dominant* eye-colours appear to be "eversporting."

We have, then, several effects which seem to be correlated: (1) dominance (in the case of eye-colours); (2) somatic variegation; (3) germinal variation; and (4) a previous breakage, together with re-attachment elsewhere, of a section of a chromosome (*i.e.* "displacement"). The cause of this strange association, except in the case of the second and third points, which are evidently only somatic and germinal aspects of the same thing, is not clear. The cases seem self-consistent and fairly consistent among each other, but as yet they do not carry their own explanation.

Some possibilities naturally suggest themselves, but the author has not yet discovered any one simple and highly plausible assumption that by itself seems capable of meeting all the essential facts. If, for example,

it be postulated that the point of breakage and re-attachment is a "weak spot," where the chromosome is liable to break again, with the resultant loss from some descendant cells of the section distal (with reference to the spindle-fibre attachment point) to the breakage, there arises the objection that the light or white areas in mottled males are nevertheless viable, although both theory and empirical findings point to the death of cells lacking such a portion of their only X-chromosome. Moreover, the individuals which start development as genetic "lights" always show some areas of deeper pigmentation. One or more radical subsidiary hypotheses would then be required, such as that the section of chromosome in question is not completely lost, but made less effective, perhaps by the loss of one component "strand," if we assume it to have been composed of two or more parallel and ordinarily identical threads each dividing before and separating at any given mitosis.

Again, instead of the section distal to the supposed weak or unstable spot becoming lost in the light areas, it might be supposed to become re-attached elsewhere, or to become attached at the same point in a different manner from before; but then this process of re-attachment would have to be regarded as likely to become reversed again to give the dark areas in the genetically light individuals. Moreover, the place or manner of attachment would then have to be supposed to be correlated with or to influence the mode of action of the genes in the attached piece, upon the protoplasm in which these genes lay.

A third possibility (which to a certain extent overlaps the first) is that the displaced chromosome segment is in some way—owing to an abnormality in its method of attachment—liable to become reduplicated, the additional strands either remaining in parallel, or else becoming detached and possibly attached elsewhere. We should then have to assume that in the segments containing the mottleds, for instance, a near-white allelomorph was present which on reduplication gave a stronger effect, much as Stern has found the mutant allelomorphs of bobbed to give longer bristles when present in larger numbers. A similar assumption would be made with regard to the Notch and other loci yielding visible differences, though the genes at these loci would not always have to be considered as mutant. This hypothesis would, to be sure, help to explain the possibility of dominant effects on eye-colour by the mere accumulation of chromatin. But it would also be necessary to predicate that the reduplicated pieces were subject to loss, otherwise the "eversporting" effect would not continue. All these peculiar assumptions subtract considerably from the strength of this hypothesis, and yet it seems on the whole the

least improbable of any specific "explanation" that can be offered pending further investigation.

Further speculation on such possibilities here seems premature, inasmuch as the experimental and cytological attack is by no means exhausted. Meanwhile, whatever the detailed explanation may be, it will be seen that the signs point to the probable inadequacy of the theory of minute independent elements ("genomeres," to use Whiting's term), segregating inside the gene. They point rather to some peculiar manoeuvres of some portion of chromatin larger than a gene which has been displaced from its original position. Hence the term "eversporting displacements." At the same time, still other possibilities—principally chemical—are not to be ruled out, and dogmatism is to be avoided at this stage of the work. As to whether or not the results have a bearing on the phenomenon of the "eversporting gene" encountered in other work, judgment may also be suspended for a while.

SUMMARY.

1. The induced visible variations involving only "point mutations" give every evidence of being the same in their nature as the spontaneous visible variations due to point mutations. Some of the former are sensibly identical with the latter, others form with the latter, and with each other, multiple allelomorphic series. Among these are certain cases of extreme lethal allelomorphs, dominant in their visible effect. Inconspicuous visibles, overlapping the normal type, are frequent. The simple point mutations induced by X-rays are stable in their inheritance, no "eversporting" cases having as yet been found.

2. Chromosome breakage, accompanied or unaccompanied by the attachment of one or more of the resulting fragments at a different locus from before ("displacement"), may lead to the production of some hypoploid and hyperploid individuals deficient or redundant for some portion of a chromosome. Instances of these, exhibiting visible abnormalities due to the gene disproportions, are described.

3. Heteroploids of abnormal appearance have also been obtained by crossing-over between chromosomes having induced inversions, and others which were uninverted or had somewhat different inversions. Through the study of such individuals, and also of individuals produced by crossing together of parents having different but overlapping translocations, it should eventually be possible to determine the phenotypic effects of hypoploidy and hyperploidy of every portion of the chromatin,

and to compare these effects with those produced by mutations of genes in the corresponding regions.

4. In general, the hypoploids are less viable than the corresponding hyperploids, the genic disproportions in the former being greater.

5. In males with heteroploidy of the *X*-chromosome, in which a part of this chromosome (including the sex locus or loci) bears to the autosomes the ratio characteristic of the male, and the other part or parts bear the ratio characteristic of the female, there is a strong tendency towards specific morphological abnormalities, and inviability. These abnormalities also occur, but to a lesser degree, in the corresponding heteroploid females. The effects are therefore due to the disproportions existing between the genes of the same kind of chromosome (the *X*), *i.e.* to intra-, not inter-, chromosomal genic disproportion. A reason for this relatively more drastic effect of intra- as compared with inter-chromosomal genic disproportion in the case of the *X* is to be found in its past evolutionary history.

6. The induced gene re-arrangements ("displacements") in *Drosophila* are usually accompanied by changes resembling those of gene mutations. Most, but not all, of these changes are lethal when homozygous. Some produce visible effects, and among the latter are both recessives and dominants, including changes at previously known loci.

7. Over a dozen cases have been found in which the visible abnormality accompanying a displacement behaves in an "eversporting" fashion.

8. An "eversporting displacement" may involve any type of re-attachment of genes: translocation, inversion, or deletion, as the case may be.

9. The genetic instability often involves both germinal and somatic tissue, giving rise in the latter to a visibly mosaic effect.

10. This mosaicism is usually most readily detected in the case of mutants which affect the colour of the compound eye, but it is not actually confined to any particular region of the body.

11. The numerous eversporting displacements called "mottleds," which involve the locus of white eye, also affect the coloration of the testicular envelope in a mosaic fashion.

12. The genetic instability in the germinal tissue in some cases results in the splitting off of a more extreme (abnormal) type, which gives few germinal variants, and a less extreme type, which retains the tendency to "split" readily as before. More than two "lines," differing in various respects, have become split off in some cases.

13. The somatic appearance of all the above types indicates that none of them has become completely stable, since all are to some extent mosaic. Upwards of fifty generations of breeding have not resulted in any completely stable types.

14. In the case of one of the "eversporting" displacements, "mottled 1," two distinct but neighbouring loci in the displaced region, that of white and that of Notch, were both rendered "eversporting" at once, and the changes involving both loci tend to occur simultaneously and in the same direction.

15. There are serious objections to the genomere hypothesis as an explanation of these "eversporting" cases—first, the fact that the displacement of an entire region is always a prerequisite; second, the fact that stable lines do not become sorted out; and third, the correlation between the variations in the two neighbouring loci, of white and of Notch, just referred to. The variations seem to be due to the behaviour of bodies which are not smaller than genes, but larger, somehow comprising segments of chromosomes.

16. The manner in which these "eversporting" chromosome segments become changed or displaced so as to give rise to mosaic tissue, is not as yet clear. The fact that some mosaic males containing a displacement involving their X-chromosome are viable indicates that the eversporting segments do not actually become lost from the cells. If they merely become changed in position, then the shift must be reversible, and it must be supposed that the position of the segments somehow affects the mode of expression of its contained genes. Types of change other than loss or positional changes are also conceivable, *e.g.* chemical changes, changes in the chromonema envelope, and especially reduplicational changes.

17. Among the "eversporting" displacements, there were included several cases of dominant eye-colour changes. There was, in addition, one dominant eye-colour change (found by Oliver) which was not visibly eversporting, but which, like the rest, involved a displacement (translocation). No cases of mutant eye colours, dominant to the normal red and involving merely point mutations, are known in this species. The cause of this association between dominance in eye colours, chromatin displacement, and an "eversporting" tendency, remains unknown, although a tendency to reduplication on the part of the displaced segment seems at present the most likely possibility.

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DESCRIPTION OF PLATES

PLATE XII.

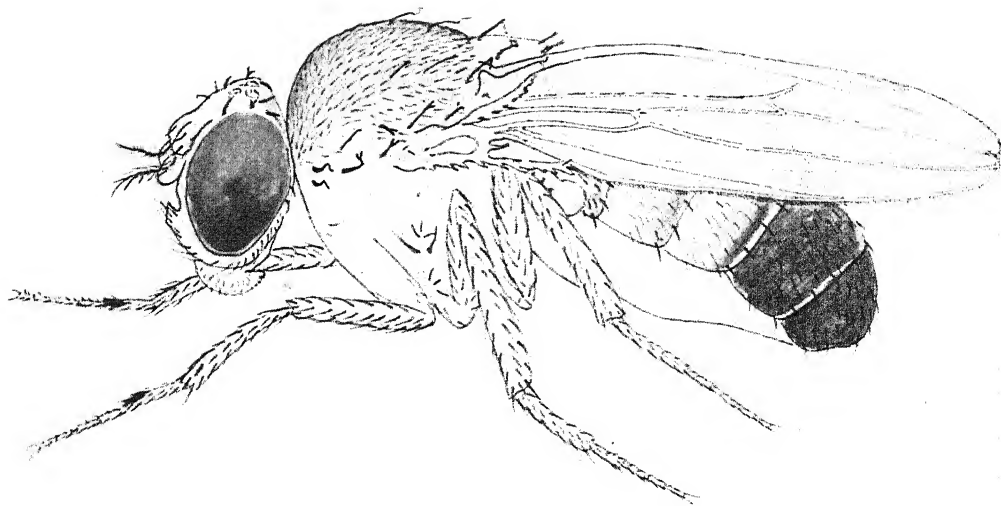
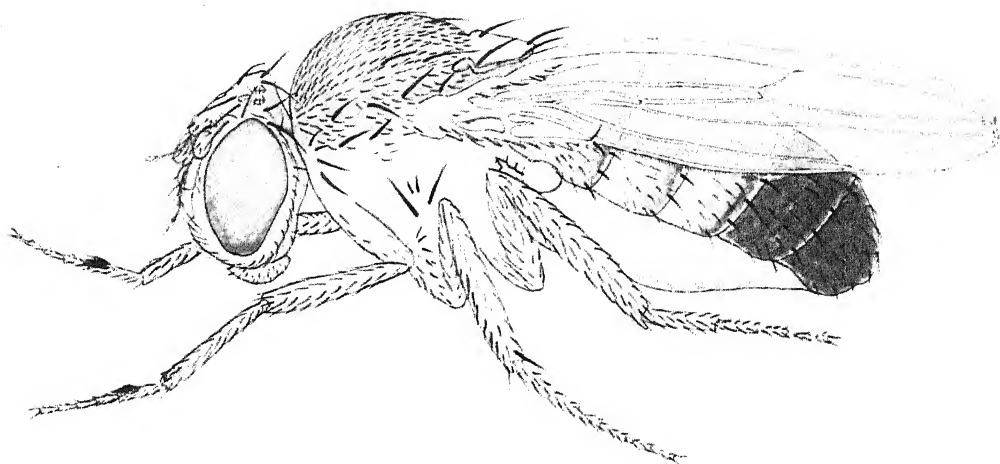
Figs. 1, 2. Above, spectacled eyes (and normal bristles)—spectacled is a recurrent induced mutation allelomorphic to the "lozenge eyes" of spontaneous origin. Below, a non-spectacled fly of the forked-bristle race from which the above spectacled arose by a simultaneous mutation from non-spectacled to spectacled and reverse mutation of forked to non-forked.

PLATE XIII.

Figs. 1, 2. Mottled 1—an eversporting "mutation" of multiple expression, accompanying a translocation. The specimen shown below is a light mottled "segregate" with the markedly Notch wings characteristic of this type; that above is a dark mottled "segregate," with the Notch scarcely discernible.

PLATE XIV.

Figs. a–h. A series of "displacements," producing dominant effects (mostly "eversporting") upon the eyes. a. Normal (for comparison). b. Punch (apparently not "eversporting"). c. Plum. d. Moiré. e. Tarnished. f. Lighter variant of Discoloured, combined with vermilion. g. Lighter variant of Discoloured (resembling Plum). h. Darker variant of Discoloured (resembling dark mottled 1).



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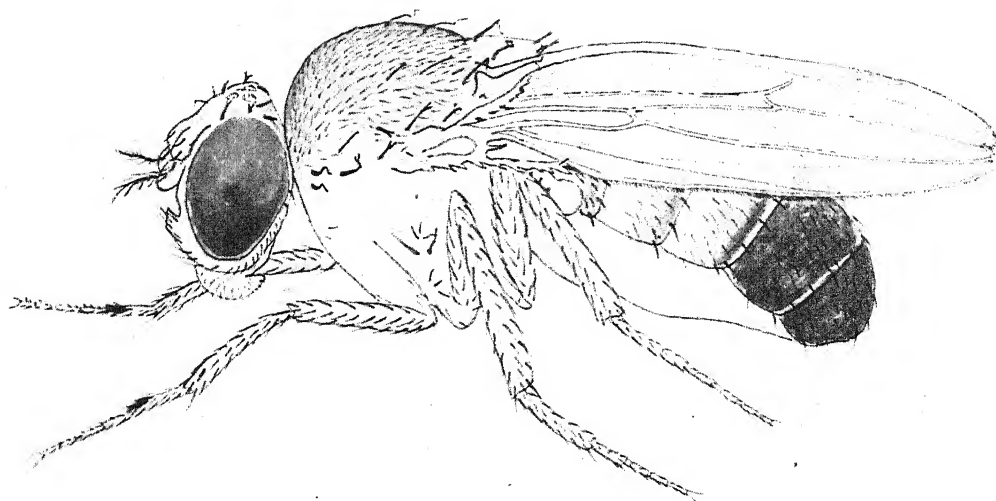
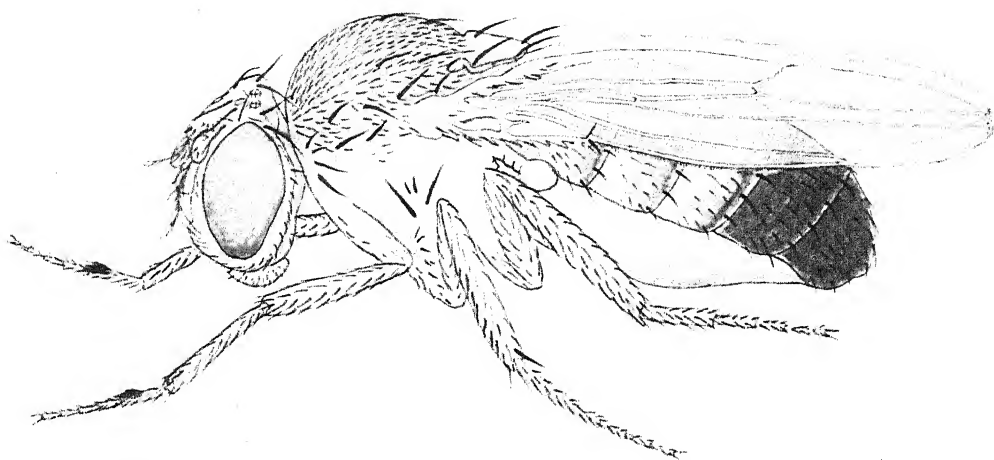
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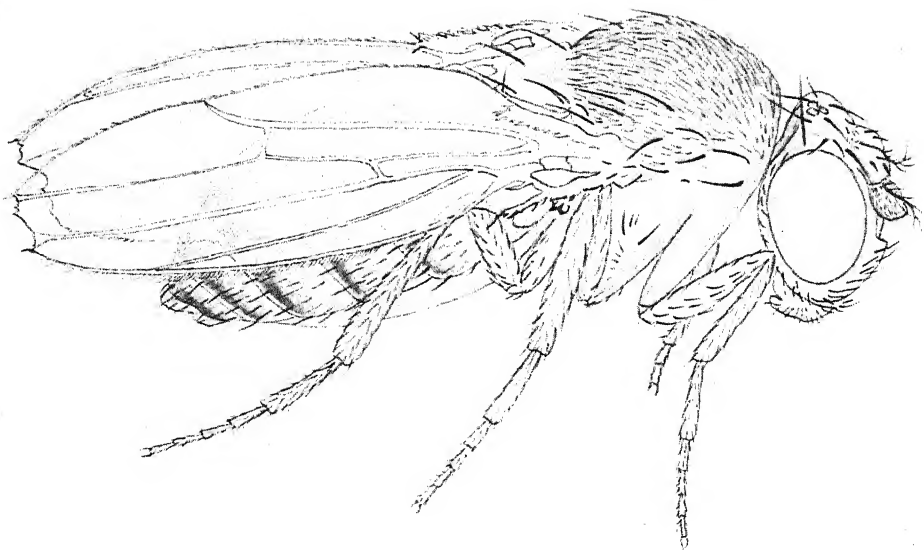
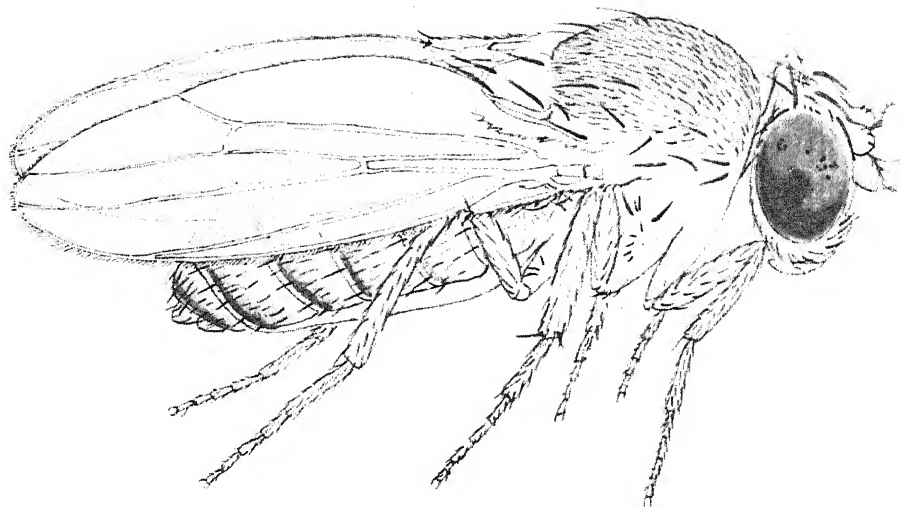
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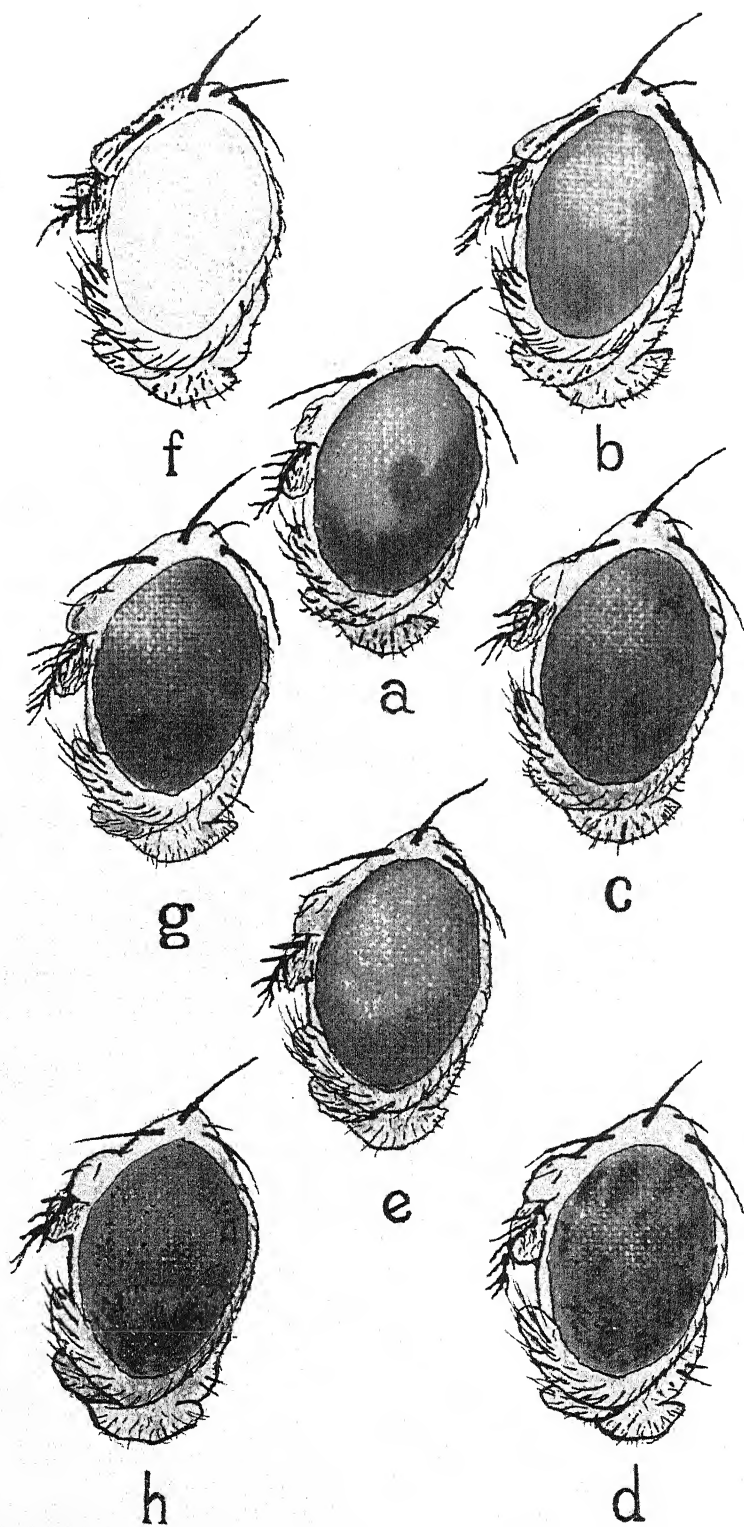
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OENOTHERA-LIKE LINKAGE OF CHROMOSOMES IN *DROSOPHILA*.

BY H. J. MULLER.

(*University of Texas.*)

(With Three Diagrams in the Text.)

I. THE PROBLEM.

THE recent masterly analysis of the *Oenothera* problem by Darlington (17), his evidence from the *Tradescantia* group (18), and the remarkable results of Blakeslee and Belling (1-7) on segmental interchanges in *Datura*, bid fair to solve, with one sweep, all of the major theoretical difficulties that were still outstanding in the *Oenothera* situation. Why do the chromosomes join in "rings"? what connection has this condition with the balanced lethals, and how did it arise? does it imply a selective segregation of non-homologous chromosomes, and if so, how? in what way do the exceptional recombinants (spurious mutants) arise, and what cytological and genetic peculiarities should they exhibit on inbreeding and on crossing? The appeal of the new theory based on mutual translocations (segmental interchanges) is almost irresistible, owing to its essentials being so simple and so accordant with known genetic phenomena, its superstructure being so logically developed, and its devious consequences being illustrated by so many facts known empirically not only in *Oenothera* but also in *Datura*, *Tradescantia*, and other forms.

But, as in the case of any new theory, no matter how compelling, it is important that it be supplemented with further observational and experimental evidence, dealing as directly as possible with the matters most fundamental to the theory. In the present instance, further evidence would be desirable, not so much to prove the possibility of occurrence of the translocations on which the theory is founded, but rather to show that, once these translocations have occurred, the resulting chromosomes will undergo segregation in the required manner, those from the same parent preferentially going to the same pole so as to form "old-combination" rather than "new-combination" gametes. For this to hold true, it is necessary that certain particular conditions should obtain which,

while plausible, have until recently been beyond the scope of genetic study.

In dealing formerly with pairs of chromosomes homologous along their entire lengths, there was no sure way of ascertaining whether the segregation of the members from one another was entirely a resultant of the disjoining forces seen to be operating primarily at the points of attachment of the spindle fibres, or whether the synapsis of the chromosomes in their other regions also played an active rôle in their later segregation, for both tendencies would in ordinary cases work towards the same consequences. In the genetic work of Bridges and Anderson⁽⁹⁾ on triploid *Drosophila*, it was indeed definitely shown that, in the first meiotic division, the impulse to separation of *homologous* strands originated only at the fibre attachment point, whereas in other regions (following crossing-over involving two strands at a time) sister strands might undergo separation instead. While this result would not in itself explain what segregational forces might be operative in cases of translocation, it might, nevertheless, be construed as at least raising a suspicion that the attachment points alone determined the direction of segregation. On the other hand, the recent results of Blakeslee with the "*B* whites" of *Datura*, as well as those of Darlington, point to the principle that, in cases of translocation, chromosomes tend to segregate not only from other chromosomes which are homologous to them in the region of their spindle fibre, but, at the same time, from those which have synapsed with them in other regions, even though the direction of the latter's migration is influenced by synapsis in still other regions with other chromosomes in turn.

It is the purpose of the present paper to present some genetic evidence that the above-mentioned processes actually occur in *Drosophila*: *i.e.* that translocations do to some extent influence the segregation of chromosomes in the manner depicted by Blakeslee and by Darlington, and so tend to result in gametes having a preponderance of chromosome combinations belonging to the parental gametic types.

II. ANALYSIS OF THE TRANSLOCATION STUDIED.

The translocation which was used in the present study is that which has been designated the "Star-Curly (*SCy*) translocation." It arose from a cross of irradiated Curly-winged males with normal females. Among the offspring, one Curly-winged individual with "Star"-like eyes appeared, which was proved on further testing to contain in the chromosome with Curly (the treated second chromosome) a mutant gene

allelomorphic to the known gene for Star eye, and similar to the latter in its phenotypic effect. Later tests showed also that the second chromosome containing *S* and *Cy* was involved in a translocation with the third chromosome derived from the treated male, since, in the male, the genes *S* and *Cy* behaved as if completely linked with the normal genes in the third chromosome.

The further genetic analysis was carried out by means of linkage tests in which the distribution of *S* and *Cy* in the second chromosome, and of the genes *ru h st p* and *ss* in the third chromosome was studied. The cross was a back-cross of females having a composition $\frac{S\ Cy}{ru\ h\ st\ p\ ss\ e}$ with multiple recessive males homozygous for the mutant genes *ru h st p ss e* (a combination called "IIIple").¹ These tests showed that *S* and *Cy* were closely linked to the third linkage group at a point about midway between *st* and *p*, i.e. in approximately the middle of the genetic map. Hence either the second chromosome was broken at some point and one of its fragments attached near the middle of the third chromosome, or the third chromosome was broken near its middle and attached somewhere on the second chromosome, or both. In all, there were fifteen single cross-overs between *st* and *p*, seven of which separated the *S Cy* class from *st*, and eight of which separated it from *p*. The number of single cross-overs between *S Cy* and each of the other genes studied in this cross was much greater.

There was one cross-over between *S Cy* and all the genes studied in the third chromosome; it showed all the mutant characters at once, namely, *S Cy ru h st p ss e*. Tests showed that in this cross-over the translocation was not present. Hence in this case there had been a crossing-over of the chromosomes of the second pair between the region containing the genes *S Cy* and the breakage point, or the point of attachment of the translocated section. It is known that crossing-over practically never occurs in the left-hand region between a chromosome containing the Curly complex and another second chromosome. The cross-over in question, therefore, occurred further to the right than *Cy* or *S*. This fact showed that the breakage point, or the attachment point of the translocated section, was not in the left-hand region of the second chromosome.

The *S Cy* translocation was later analysed cytologically by Painter,

¹ *ru*=roughoid eyes, *h*=hairy body, *st*=scarlet eyes, *p*=pink eyes, *ss*=spineless body, *e*=ebony body. The symbol L^2 , used later, designates the dominant "Lobe² eyes," in the second chromosome.

and the results, both of the cytological and the genetic analyses, have been presented in papers by Painter and the present author (33, 37). The cytological analysis showed conclusively that one of the long autosomes had become broken at a point near the attachment of the spindle fibre, normally located centrally in these long chromosomes, and that the fibreless fragment (nearly one-half of the chromosome) had become attached to (or very near to) one end of the other long autosome. Since the genetic evidence had already shown that the third chromosome had become attached, or else broken, with one of its pieces attached, near its middle, it was evident that the chromosome seen to be broken near its middle must be the third chromosome. Therefore the other chromosome, having the piece terminally attached to it, must be the second chromosome, and the end bearing the attached piece must correspond to the "right-hand" end of the genetic map, the left-hand region having been shown genetically not to contain the point of attachment (or of breakage).

The abundance of cross-overs between the various genes studied in the third chromosome, and between them and *SCy*, proved further that the displaced fragment must have become attached by its broken end, not by its originally free end. If the latter had been attached, it can readily be seen that all single cross-overs between the attached section and the homologous section in the unbroken third chromosome would, on the one hand, result in double chromosomes consisting of an entire second and an entire third chromosome attached together, each still possessing its own fibre-attachment point (such a double chromosome should often be pulled in two directions at once during mitosis, and so become broken, or else tend to falter between the two poles) and, on the other hand, in short fibreless fragments of chromosome III that would not be transportable at all at mitosis. Numerous single cross-overs were, however, obtained. Some of these were tested and found to contain the translocation in its original form.

Crosses were next undertaken with a view to obtaining definitive genetic evidence that it was the third chromosome which was broken, and to ascertain whether it was the right or the left arm which had become attached.

The most crucial genetic criterion of breakage is in itself simple enough. If a certain chromosome is broken, male gametes can be formed which contain one segment of this chromosome but not the other; if it is not broken, such gametes cannot be formed. These male gametes can, however, result in viable zygotes only if the female gametes which they

fertilise contain the complementary segment (and not the identical segment) and if, in addition, one of the uniting gametes contains an entire chromosome homologous to the broken one. It was therefore necessary, for the present test, that both parents contained the translocation, and that both the segments of the affected (broken?) third chromosome in the male were differentiated from the corresponding segments in the female by means of "identifying genes." The crosses to "IIIple," previously referred to, had already provided material in which, owing to crossing-over, both segments of the affected third chromosome contained recessive mutant genes serving to differentiate them from the corresponding segments in the original stock of translocation. Since these mutant genes are recessive, it was necessary for the non-affected (entire) chromosome of one or both parents to contain them also; otherwise they would be unable to manifest themselves in the offspring and could not serve as visible "identifiers" (markers). The various crosses depicted in Table I all exhibit these required features: the presence of the translocation in both parents, the possession of different markers in the two parents in the case of both segments of the affected third chromosome, and the presence of the recessive markers in the non-affected (entire) third chromosome of one or both parents. The last cross is less clean-cut in its results than the others because the female instead of the male was caused to be heterozygous for certain of the identifiers, thus allowing crossing-over somewhat to obscure the picture.

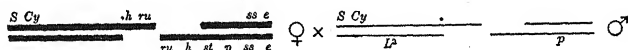
The "first cross" was so framed as to reveal not only whether the third chromosome was broken, but, in case it was broken, whether its left or its right segment was attached to the second chromosome. The male parents here had the composition $\frac{SCy}{L^2} \cdot \frac{\quad}{p}$ and

the female parents had the composition $\frac{SCy}{ru} \cdot \frac{hru}{st} \frac{sse}{p s s e}$.

(The method of breakage and attachment proved by the results of this cross is here assumed true in representing the composition of the parents, the point of attachment being indicated by a dot above the line representing the chromosome.) If, now, the affected third chromosome of the male, containing the dominant genes, is really broken, some offspring should inherit a part of the dominant genes of their father, namely, those in the free segment, without the complementary part, in the translocated segment. Those of this derivation which lived would, however, receive a translocated segment from their mother and this segment would contain recessive genes. They would, if viable, receive in addition

an entire third chromosome. Except in cases of "non-disjunction" (*vide infra*), this would come from their mother, and thus would contain all the recessive genes. Hence this class of offspring would show the dominant characters of the free fragment from the father, and the recessive characters of the translocated fragment from the mother. The ten flies of type $S\ Cy\ L^2\ ru\ h$ obviously represent this class, and it is therefore evident, (1) that the third chromosome is really broken, and (2) that the left-hand segment (containing the recessives ru and h from the mother) is the translocated, attached segment. The presence of L^2

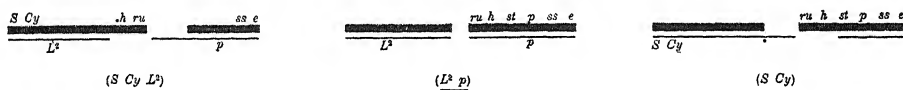
Diagram I. Viable "regular" combinations producible by the cross:



Chromosomes belonging to or derived from P_1 female are shown with heavy lines; those of P_1 male with light lines. Phenotypic characteristics of zygotes are shown in parentheses below their respective genotypes, unique phenotypic combinations being underlined twice.

(Cross-overs between st , p , and breakage point in ♀ are disregarded.)

Old combinations in eggs and sperm:



New combinations in eggs and sperm:



(Points of attachment indicated by dots above or below lines.)

Count of phenotypes from above cross: $S\ Cy\ L^2$, 26; $L^2\ p$, 26; $S\ Cy$, 48; $S\ Cy\ L^2\ ru\ h$, 10; all others, 0.

is a check on the fact that their father provided them with an unaffected second chromosome, their mother providing the $S\ Cy$; this agrees with the fact that ru and h were able to manifest themselves.

On the other hand, in all offspring failing to show L^2 , the affected second chromosome of the father, with its attached piece of the third, must be present. None of these (the $S\ Cy$ non-Lobe² flies) show ru and h , and it could be concluded from this fact alone that the attached piece must contain the normal allelomorphs of these genes, *i.e.* it must be the left-hand segment. The experiment thus provides a double criterion of which region became translocated.

III. THE EVIDENCE FOR SELECTIVE SEGREGATION.

Although the original objectives of the test were attained merely by a determination of which of the crucial classes were present and which absent, it was of interest also to examine the comparative numbers in which the different classes occurred. Such examination revealed at once a surprising deficiency of the class *S Cy L² ru h*, the class which had resulted from a union of two gametes both of which contained a "re-combinational" grouping of chromosomes—one gamete having had the affected second with the unaffected (entire) third, and the other having had the unaffected second with the affected (free fragment of the) third. In order to determine whether these numerical relations were significant, it was necessary to know (1) that they were not due to the swelling of the supposedly old-combination classes by the inclusion in them of flies of similar phaenotype that had really been produced by a different process—"non-disjunction," and (2) that the numerical disproportions were not due simply to lower viability on the part of the more multiple-mutant combination.

The question of possible "non-disjunction" in cases of translocations is of considerable importance. The term is used here solely to mean non-disjunction of those portions of the chromosome to which the spindle fibre is attached. All possible "non-disjunctional" combinations of the second and third chromosomes in this experiment which would be viable have therefore been shown in Diagrams II and III. Diagram II concerns itself with non-disjunction involving the chromosomes of only one kind, the second *or* the third, and Diagram III with cases involving both second and third at once. In no case, of course, is a non-disjunctionally derived zygote viable unless non-disjunction occurred in the origination of both paternal and maternal gametes, the unions involving complementary combinations. Yet if non-disjunction occurs with a frequency at all comparable with that of disjunction (segregation), such zygotes should have occurred in counts of the size here observed, for the viable offspring resulting from the various kinds of disjunction also were limited to the unions involving complementary combinations, and numbers of these were found. If, on the other hand, viable, non-disjunctional combinations do not occur frequently enough to be detected in the present experiment (in those classes in which they would be identifiable), they cannot be numerous enough to swell materially the numbers of the apparent old-combination classes either—which was the question at issue.

It will be seen from Diagram II that of the various viable classes

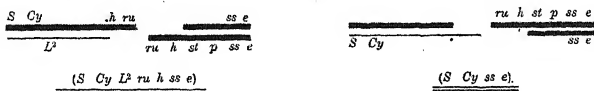
Diagram II. Viable non-disjunctional combinations involving one pair of long autosomes, producible by cross shown in Diagram I:

(System of representation as before.)

Excess of II in eggs; deficiency of II in sperm:



Excess of III in eggs, deficiency of III in sperm:



Deficiency of II in eggs; excess of II in sperm:



Deficiency of III in eggs; excess of III in sperm:

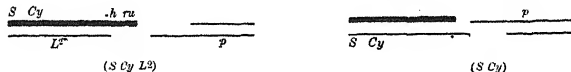
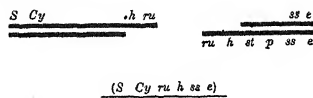


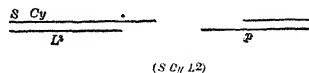
Diagram III. Viable non-disjunctional combinations involving both pairs of long autosomes, producible by cross shown in Diagram I:

(System of representation as before.)

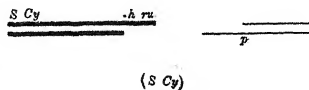
Excess of II and III in eggs; deficiency of II and III in sperm:



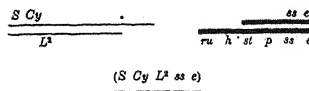
Deficiency of II and III in eggs; excess of II and III in sperm:



Excess of II and deficiency of III in eggs; deficiency of II and excess of III in sperm:



Deficiency of II and excess of III in eggs; excess of II and deficiency of III in sperm:



formed by non-disjunction of one kind of chromosome, three would be phaenotypically different from all other classes, and hence separately recognisable as such. One of these three classes involves non-disjunction of the second, the others non-disjunction of the third chromosomes. None of the non-disjunctional classes not separately recognisable would be expected to occur in greater numbers than some of these recognisable classes. Yet no flies belonging to the recognisable classes were found among the total of 110 individuals. It is therefore safe to conclude that, in this and other experiments with this translocation, there can be no material distortion of ratios due to the inclusion of non-disjunctional cases with other classes resembling them phaenotypically. Inasmuch as none of the simpler non-disjunctional cases were found, it is only to be expected that none of the recognisable classes of Diagram III—representing non-disjunction of all the long autosomes at once—would be found either, since these should occur even more rarely; and as a matter of fact none were found.

Flies phaenotypically like those in the classes of Diagram I may therefore be considered as really belonging to them. It will be noted that in this "first cross" three of these five classes, including one new and two old-combination classes, were distinguishable, while the other two looked alike (*SCy*). Each of the two recognisable old-combination classes (containing twenty-six individuals) was significantly larger than the new-combination class (of ten individuals), but the mixed *SCy* group (of forty-eight individuals) was larger than it would have been if the old- and new-combination classes composing it had each been no larger than were the other old- and new-combination classes. This *SCy* group, be it observed, was the most nearly normal group, phaenotypically. Differential viability evidently produced appreciable effects on these numbers, and it is conceivable that the one recognisable new-combination class, *SCy L² ru h*, was numerically small simply on this account, since it contained more mutant genes than did any of the others.

In order to obtain light on the question just raised, viz. whether the small size of the new-combination class was due only to lower viability, other crosses were therefore undertaken, shown in the second to fourth rows of Table I. These were so framed that in their progeny all the viable classes (disregarding non-disjunction) should be separately recognisable, and that the new-combination classes should not, on the whole, show more mutant characters than the old-combination classes. In most cases, in fact, the reverse was true, the old combinations showing more mutant characters. Moreover, in any one experiment the different old

TABLE I.

Crosses illustrating selective assortment of chromosomes.

(Both male and female parents contain the "Star-Curly translocation.")

Composition of ♀ parent	Composition of ♂ parent	Count of offspring			Genetic ratio of old:new combinations	√Ratio— old:new
		Old combinations	Old + new combinations	New combinations		
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y L^2$:26	SC_y :48	$SC_y L^2 r_u h$:10	2.6:1	1.6:1
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$L^2 p$:26				
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y r_u h (s_i p) s_s e$:46				
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$r_u h (s_i p) s_s e$:43		$SC_y r_u h (s_i) s_s$:19	3.15:1	1.8:1
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	SC_y :81		$SC_y (p) s_s e$:17		
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y r_u h (s_i p) s_s e$:12				
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$r_u h (s_i p) s_s e$:12		$SC_y r_u h (s_i) s_s$:6	2.36:1	1.5:1
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y e$:15		$SC_y (p) s_s e$:5		
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y r_u h s_i (ps_s) e$:25				
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$r_u h s_i (ps_s) e$:48	n.c.	$SC_y r_u h s_i (ps_s) e$:10		
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y (ps_s) e$:47		$SC_y e$:8	3.2:1	1.8:1
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$r h s e$:1				
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$r_u h s_i p e$:4		$SC_y r_u h s_i s_s e$:6		
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y p e$:3	c.o.	$SC_y r_u h s_i p e$:3	3.0:1	1.7:1
$SC_y \cdot hr_u$	$SC_y \cdot L^2$	$SC_y s_s e$:2				

(n.c. = non-cross-overs; c.o. = cross-overs.)

(Points of attachment indicated by dots placed above them.)

Genes shown in parenthesis were present only in some cases, owing to the occurrence of crossing over between them and the point of breakage.

Average

combinations showed considerable diversity, as also did the new combinations, and hence could serve as checks against each other's viability. Examination of the results in the table shows that while there is in fact clear evidence of differential viability, shown by the numerical differences between different old-combination classes, nevertheless, the numerical superiority of the old- over the new-combination classes, quite apart from differential viability, is unmistakable. In no instance was even the highest new-combination (non-cross-over) class more than half as great as the lowest old-combination (non-cross-over) class, and the ratios of old to new combinations derived from the different crosses were in good agreement (see next to last column, Table I). There could then be no doubt about the reality of the phenomenon. The conclusion therefore follows that, at the segregation division, the chromosomes are actually more apt to be aligned in the old combination, and that assortment is not random as it is in the case of completely non-homologous chromosomes.

It will be of interest to make some calculations regarding the strength of this selective tendency on the part of the segregating chromosomes. In so doing, we must remember that the observed zygotic classes in each case are derived from the union of two gametes that were complementary in regard to their translocations, zygotes having deficiency or excess of the chromosome-regions involved being quite inviable. These complementary classes are, in each case, both derived from mother cells that have the same kind of chromosome arrangement, and, by their division, give rise simultaneously to the two respective complementary classes. If, now, the number of mother cells having the old combination of chromosomes be represented by a and the number having the new combination by b , both in the case of the male and of the female gametogenesis, then the numbers of zygotes derived from the union of two old-combination gametes will be to the number of those derived from the new-combination gametes as a^2 is to b^2 . What we observe is the ratios which these zygotic classes bear to each other. To get the gametic ratios from them in experiments of this type, we may simply take the square root of these ratios. Applying this principle to the lumped data from all the crosses in the present experiment, we find that the chromosomes were about 1.7 times more likely to give rise to old-combination gametes than new-combination ones. There is doubtless some error here due to differential viability, but there can be no doubt about the substantial correctness of the results. It should be noted also that if the tendency to form new combinations is different in the two sexes, then the value 1.7 will

not apply to either sex alone, but the gametic ratios in the two sexes together will have this value as their geometric mean.

These results evidently furnish a clue to the findings of Cleland and others in the *Oenothera* case, by providing the missing mechanism whereby the groups of chromosomes would undergo a selective form of segregation, or "linkage," and it was planned to present the case from this point of view. While the preparations for such a paper were under way, Darlington's first paper on the matter (17), appeared, and the recent papers of Blakeslee (6, 7) giving evidence for selective segregation in *Datura* came to the writer's attention. Shortly after this came Darlington's second paper (18) and the related papers by Meurman (26) and by Håkansson (24). The present case thereby assumed the form of a confirmation of Blakeslee's and Darlington's work, although it had been intended to serve as the basis for the independent establishment of the principle involved, and for the explanation of the *Oenothera* chromosome "linkage."

In the present case, the new combinations are considerably less numerous than the old combinations, and yet they do occur fairly frequently, whereas in the case of *Oenothera* it seems likely that they occur so seldom that they may there be classed as "abnormalities." It is quite likely, however, that there would be considerable differences between different organisms in respect of the strength of the tendency for chromosomes partly homologous, but non-homologous at the points of their spindle-fibre attachments, to enter different gametes. Among other things, much would depend on the precise method of drawing apart of the chromosome strands after synapsis, and the resultant final modes of attachment at the time of segregation. This behaviour is known to vary greatly from one organism to another. Biterminal attachments (chiasmata near both ends), as in *Datura* and *Oenothera*, would be particularly conducive to a strong influence of translocations upon the direction of segregation, as Darlington has pointed out.

The morphology of the translocations also would be important in determining the amount of their influence on the positions of the chromosomes at reduction. Thus, the Star-Curly translocation was large, and included terminal region; it would therefore be expected to be particularly potent in this way (at any rate, for a *Drosophila* translocation). The translocated piece is, however, even in this case, barely half as long as the chromosome to which it is attached; if it could have been as long as or longer than this, it would probably have been still more potent in directing chromosome segregation. In this connection, the results

are of interest which are obtained when a large piece of one of the long autosomes is attached to the tiny fourth chromosome (see discussion at the end of this paper). When a portion of the recipient chromosome is itself broken off, and either attached to a third chromosome (double translocation) or to the first, broken chromosome (mutual translocation), this, too, would serve to make the attached piece longer, relatively to the recipient (spindle-fibre-bearing) piece. Mutual translocations ("segmental interchanges")—which we now have evidence are produced by irradiation in *Drosophila*, in addition to the simple and the double (as above defined) translocations—should theoretically be particularly effective in preventing the formation of new-combination classes. At the other extreme, we have such a translocation as the "translocation 1" found by Bridges in 1923(8) in untreated material. This involves only a small fraction of the second chromosome, which is somehow attached to the third chromosome in a position which the genetic evidence indicates to be non-terminal. The studies of Muller and Settles(34) show that in the case of this translocation as many new-combination as old-combination gametes are produced.

Attention should be drawn to the fact that, in cases of translocation, it is not the old- *versus* new-combination classes of gametes *per se* which are caused to be produced in unequal numbers, but the genetically normally proportioned ("balanced" or monoploid) *versus* the genetically disproportioned ("unbalanced" or heteroploid) classes of gametes. Thus, in our case, individuals which themselves are the result of union between two complementary new-combination gametes (one having had the normal second and deficient third, the other the translocation-bearing second and normal third chromosomes) should, like the other individuals bearing this translocation, produce an excess of normally proportioned gametes (*i.e.* gametes having a normal second and normal third, or having a translocation-bearing second and a deficient third chromosome), although such gametes are here to be classed as "new combinations." In this respect, this inter-chromosomal "linkage" gives results fundamentally different from those of the linkage between genes in the same pair of chromosomes, and a genetic criterion of it would thus be provided.

IV. THE SITUATION IN *OENOTHERA*.

Before closing, a brief review may be made of some of the features of the *Oenothera* situation which are more especially related to the points just discussed. De Vries(19) showed that some of the *Oenothera* species

other than *lamarckiana* were in a state of enforced heterozygosis, and that homozygous individuals could never be formed, due to the male and female gametes of opposite types being functionless (or inferior in their functional capacity, as Renner has amended the matter for some of the cases). *Oe. lamarckiana* also was shown by de Vries's results to be heterozygous, since on crossing with other species it produced two kinds of hybrids simultaneously, but its male and female gametes of both types were functional. Renner, in 1914(38), on the basis of seed-mortality studies, concluded that its enforced heterozygosis was probably due to inviability of the homozygotes, and in 1917 (40) he suggested that the segregating genetic materials formed two contrasting "complexes," between which interchange might sometimes occur, with the resultant production of some "mutant" types.

On the basis of an analysis of the seemingly similar case of beaded wings in *Drosophila*, the present writer, in 1917 (27), independently proposed an essentially similar hypothesis, that of "balanced lethals," to explain both the enforced heterozygosis and the apparent mutants (exclusive of those of heteroploid or polyploid composition). This hypothesis was conceived in terms of the ordinary gene and chromosome theory, as dealt with in *Drosophila*, the lethals being regarded as ordinary genes, and the "mutants" as due to ordinary genes and groups of genes, lying in definite loci in the chromosomes and subject to the same fundamental rules as other genes. It was shown that the very existence of a state of enforced heterozygosis due to balanced lethals would tend to result in the accumulation of an ever-increasing number of heterozygous recessive mutant genes, linked with the heterozygous lethals. And, due to occasional exchange between the linkage groups, some of these genes, and groups of genes, would emerge into the homozygous condition, thus giving rise to certain definite types of "sports" in small but fairly regular numbers, generation after generation. The linkage thus postulated for *Oenothera* was not at that time thought of as unorthodox; it was thought of as existing between different genes lying in the same pair of chromosomes, as in the imitation case that had been artificially synthesised by the use of the Beaded stock, and the exceptions to the linkage (exchanges) were hence thought of as due to ordinary crossing-over. But whether or not this were true, the effect of the balanced lethals, in tending to prevent the frequent manifestation of the mutant genes that were linked with them, would remain undisturbed; so, too, would their effect in leading to the accumulation of an increasing battery of such genes. It was also shown how such balanced lethals might have

become established through the higher initial survival rate of some heterozygous individuals as compared with the homozygous ones (27, 28)¹.

It was soon afterwards found by Shull (49, 50) that linkage of visible recessive genes and lethals did occur in *Oenothera*, and he accordingly supported the explanation of the "mutants" on the basis above given. Sterling Emerson (20), on the other hand, called the theory into question because of the fact that the frequency of recombination of the genes studied varied radically in different crosses, and the fact that the same lethals were not always present. Some such variation was, however, to be expected, even on the balanced lethal theory in its specific original form. For, on that theory, it was necessary to assume (as had been found to be true in the Beaded case) that genetic inhibitors of interchange (now known to have been inversions in the Beaded case) were present, and it is well known that such inhibitors result in very different interchange frequencies according to the types of companion chromosomes with which the chromosome carrying the special interchange inhibitors is associated, chromosomes having like inversions presenting no hindrance to crossing-over². Further work of Renner (46, 47), how-

¹ Renner's analysis of the *Oenotheras* shows that although several species agree in having enforced heterozygosis, due to zygotic or gametic lethals (or weakening genes) or both, the same lethals are rarely present in two different species. Hence it is probable that the cause which brought about the enforced heterozygosis considerably antedated the lethals. This point, in connection with Darlington's theory for explaining the evolution of the systems of translocations by their value in enforcing further heterosis, suggests an amendment to the present writer's rather too specific original hypothesis concerning the origin of the balanced lethals in the *Oenotheras*. I had postulated that heterozygosis might have been at a premium in the *Oenotheras* owing to the presence of some particular dominant gene in the original population which, when heterozygous, was advantageous (i.e. had enhanced survival value) but which, when homozygous, was directly lethal, like Beaded in *Drosophila*. Any lines in which, besides this lethal, a balancing lethal arose, would then possess an additional advantage over other lines. It is now evident that the individuals homozygous for the advantageous dominant gene, or genes (as we may now say), need not in the first place have been completely lethal, but only relatively feeble as compared with the heterozygotes. Further, their weakness need not have been directly due to the homozygous condition of the above dominant gene or genes, but may instead (or in addition) have been due to the manifestation of genes "for weakness" linked with them, that became homozygous simultaneously. This would practically reduce the original genetic situation in these organisms to that conceived of in Jones's theory of heterosis—the idea which Darlington employs to account also for the further evolution of the translocations. It is quite possible, however, that there was originally some one particular dominant gene of especial value (when in heterozygous combinations), which furnished the starting point for the simultaneous balanced lethal development in all the types; this can probably be decided best by studies on the homology of the chromosomes in which the lethals of various species lie.

² Similarly, on our theory in its present form, the hindrance to inter-chromosomal

ever, indicated that there was a considerable number (7) of genetically separable components (some of these perhaps further subdivisible) which might on occasion behave as completely independent in inheritance, and on other occasions be closely linked; and Renner inclined to the view that this linkage probably involved associations between entire chromosomes—a conclusion at which Cleland arrived as early as 1921 on the basis of his cytological observations.

Gates⁽²¹⁾ had first called attention to the tendency of the *Oenothera* chromosomes to form groups at the reduction division, but Cleland⁽¹⁰⁻¹⁵⁾, followed by Oehlkers⁽³⁶⁾, Håkansson⁽²³⁾, and others, found that the groupings were specific and varied from species to species, including in *Oe. muricata*, for example, the entire chromosome complex in the zig-zag "ring" formation. As is now well known, Cleland suggested that the non-homologous chromosomes in a group were linked to one another, somewhat, in effect, as the genes in a single chromosome are linked in ordinary cases. At that time, however, such a postulate seemed hazardous, for, as Shull⁽⁵¹⁾ among others has pointed out, the proof of a tendency for chromosomes to cluster together in ring formation is very far from the proof of a tendency for these chromosomes to cluster only in such a way as to form the old combinations in segregation. The chromosomes of pair **Aa** may indeed have a tendency to come into contact at their ends with those of pair **Bb**, but, *a priori*, this does not mean that **A** tends to go to the same pole as **B** rather than with **b**, while **a** tends to go to the pole with **b** rather than with **B**; and it is precisely this point which would need to be proved before such a theory could be regarded as valid. Nevertheless, the later results of Oehlkers⁽³⁶⁾ and of Cleland in association with Oehlkers⁽¹⁶⁾, brought cogent evidence to bear on the question at issue, by showing that where there was more clustering of chromosomes, genetic evidence of linkage was more readily to be found. Renner's work^(46, 47), taken in connection with the cytological findings, clearly pointed in the same direction.

Meanwhile, evidence had been obtained by Blakeslee⁽⁴⁻⁷⁾, Belling^(1, 2), and their associates⁽³⁾ showing that in *Datura* a ring-like clustering of chromosomes was produced at the time of segregation, in cases in which interchange had taken place, presumably, between originally non-homologous members. For in this way the completeness of homology between the members of individual pairs might be broken up; chromosomes might be formed which were, in one region, homologous to one

interchange caused by the translocations is removed when the chromosomes from the two parents carry the same translocation.

another, and, in another region, homologous to different chromosomes, thus owing a kind of dual allegiance. Groups could accordingly become formed in which it was no longer legitimate to speak of pairs such as **Aa** and **Bb**, but in which each individual member was distinctive and had its own definitive place and relationships in a cluster. Such a situation, which is rather different from that pictured originally by Cleland for *Oenothera*, or, at any rate, considerably more specific in its explanation of the clustering, might much more readily be conceived to result in determinate assortment of chromosomes than would a condition in which the pairs were strictly homologous, provided the principle were correct that local regional conjugations of chromosomes, such as had been proved by Belling to occur consistently in cases of partial homology, influenced also the migration of the chromosomes as a whole. Blakeslee (6, 7) adduced evidence of such selective segregation, in the finding that abortive pollen are not necessarily formed by the plants having the "rings."

The applicability of these *Datura* findings to the explanation of the chromosome "rings" in *Oenothera* has been pointed out by Blakeslee (7) in his recent paper, and it was independently discussed in detail by Darlington (17) in the work referred to at the beginning of the present article. Darlington, in this paper, also called attention to the genetically proved cases of translocation in *Drosophila* as indicating the generality of the translocation phenomenon. He then proceeded to show how a series of translocations, such as would be required for the ring structure, might arise, and what its survival value would be, once a condition approaching that of balanced lethals had become established. The results of crossing types with supposedly different translocation systems were also shown to conform to expectations. Darlington furthermore pointed out that occasional new translocations would bring about exceptions to the previous chromosome linkages, and so make homozygosis in certain chromatin elements possible. This would result in "mutant" types phaenotypically and cytologically different from the original, and in these "mutants" the gene linkages and the visible chromosome groupings would be different from those of the parent form, just as found by Renner¹. Since ordinary crossing over probably can occur also, it, too,

¹ Unless, however, the breakages occurred at loci identical with those of the previous breakages, the partially homozygous types that "emerged" would be genetically "unbalanced" and might show corresponding phaenotypic disturbances; these would differ on different occasions, according to the exact locus of the breakage. Mere interchange of sections of the ring might account for some "mutants"; these, although "unbalanced," would not necessarily have an abnormal number of chromosomes, but they would show more individual pairing of chromosomes than the parent form.

could lead to "mutants"; and these latter might differ phaenotypically but not cytologically, or in their linkage relations, from their parents. Further, the frequent occurrence of trisomic types can be more readily understood as a consequence of the occasionally imperfect action of the translocations in determining the direction of segregation of the chromosomes.

The papers of Meurman⁽²⁶⁾ and of Håkansson⁽²⁴⁾, and the later paper of Darlington⁽¹⁸⁾, lend further support to his general theory. As mentioned previously, the results in the present paper, combined with a consideration of those obtained by Blakeslee and Belling, had independently led the present author also to the conclusion that the chromosome "rings" of *Oenothera* owed their existence to translocations, and (through the mechanism of autonomous conjugation of chromosome parts) involved a real linkage between chromosomes derived from the same parent. The fact that these conclusions were reached in this way should serve to make them even more corroborative of Darlington's theory than if they had been obtained with the intention of simply confirming it.

In recapitulation: it will be seen that the present theory does not discard the earlier theories to which reference has been made, but builds upon them as bases and incorporates their essentials into itself, meanwhile reaching far beyond so as to explain phenomena that were little realised when the earlier theories were first put forward. Balanced lethals are an essential part of the scheme, so, too, is the linkage with these lethals of heterozygous genes or gene complexes affecting visible characters, and also the occurrence of exceptions to this linkage which enable these previously heterozygous genes or gene complexes to appear in homozygous condition, thus simulating mutants. This linkage is, however, far more widely spread through the chromatin than had at first been imagined, including an inter-chromosomal linkage dependent on segmental interchanges, and thus the essentials of the theories of Cleland, Renner, and others, as well as the *Drosophila* investigations, are brought into one harmonious picture. In this connection it may be mentioned that Renner⁽⁴⁷⁾ had inclined to the view that the inter-chromosomal associations illustrated some new fundamental principle, possibly chemical in nature, that was not illustrated in the operations of the *Drosophila* germ plasm—a position which must now be abandoned. Moreover, as Darlington has made clear, the chromosomal interchanges are not to be regarded merely as an unrelated set of curious phenomena, accidentally superimposed on the other peculiarities, since, given the

balanced lethals and the phenomenon of heterosis, the accumulation of such translocations would present an advantage for the race by virtue of its making widespread heterozygosis obligatory. The existence of this condition would then, in its turn, by "widening" the linkage with the lethals, tend to result eventually in still more heterozygosis (through the accumulation of more recessive mutations), and so the spurious "mutants" caused by exceptions both to the inter- and to the intra-chromosomal linkages should, with time, become increasingly extreme when not actually lethal in themselves. Checks to crossing-over, *i.e.* inversions, might, however, be expected to arise in time, which would tend to prevent the occurrence of the intra-chromosomal interchanges.

While the whole group of phenomena just discussed would tend to assemble itself in a race of monoecious plants in which the pre-requisite conditions noted above first existed, it should be observed that in animals such a genetic situation would rarely be expected to arise, owing to the fact that animals are commonly biparental. As Haldane's work on the effects of selection shows, balanced lethals could scarcely be expected to become established in organisms which are not self-fertilized, and in view of this the associated heterozygous translocations would not be expected either, except in artificially synthesised races. This corresponds with the cytological evidence to date, for chromosome "rings" have thus far been found, in natural races, among plants only.

V. NON-DISJUNCTION INVOLVING THE FIBRE-ATTACHMENT POINTS.

The fact that no non-disjunction (involving fibre-attachment points) was observed in the chromosomes in which it could have been detected is worthy of separate attention. It indicates that even though a chromosome is influenced in its migration by some other chromosome which is, in part, homologous to it, it nevertheless will not easily be forced towards the same pole as a third chromosome to which it is homologous in that region which includes the point of fibre attachment. Other things being equal, it seems to be, in *Drosophila*, the latter region which has the greater weight in deciding the direction of migration¹, although the chromosomes do tend in general to become arranged in such a way that other homologous elements also separate.

When, however, the homologous region that includes the attachment point is very small as compared with the conjoined region having a

¹ This effect in the Star-Curly case was probably not due to the smaller size of the translocated as compared with the free fragment of chromosome III, since the effect was pronounced and the size difference was relatively slight.

different affinity, the effectiveness of the tendency for such homologous regions to separate from one another is diminished, so that non-disjunction may then occur more often. Evidence of this is to be found in other work of the author and of Painter with translocations produced by X-rays, involving a large portion of the third chromosome attached to the fourth chromosome. In such a case the direction of segregation of the fourth chromosome that has the large piece of the third attached to it seems to be determined mainly by the position of the large homologous section of the third chromosome (still attached in its original position in the normal third chromosome) rather than by the other fourth chromosome. The latter, then, often undergoes non-disjunction with its homologue (33, 37).

A situation like that just described probably finds little counterpart in *Oenothera* where the chromosomes are of fairly equal sizes, and the direction of migration seems almost completely determined by the terminal synaptic relationships. That there is, however, some tendency in *Oenothera* towards non-disjunction in this sense is proved by the not infrequent occurrence of trisomic individuals.

SUMMARY.

1. Data are presented which show that, where a large portion of the third chromosome of *Drosophila* is attached to the second chromosome, the directions of migration of the free fragment of the third chromosome and of the homologous third chromosome of normal structure are decidedly influenced by or correlated with the position and direction of migration of the second chromosome bearing the translocated piece of the third, with the result that nearly twice (1.7 times) as many gametes are formed bearing normally proportioned ("balanced") chromosome combinations as those bearing disproportioned ("unbalanced") combinations. The assortment thus tends to be determinate rather than random.

2. This shows that linkage between chromosomes, tending usually to result in the formation of "old-combination" (or, more correctly, normally proportioned) types of gametes, can occur somewhat as Cleland postulated for *Oenothera*, but that such linkage follows as a consequence of previous interchange of material between non-homologous chromosomes in the manner indicated by Darlington and by Blakeslee and Belling. The present finding, therefore, lends genetic evidence in support of Darlington's theory of the *Oenothera* phenomena.

The latter theory, in its turn, is considered as including a confirmation and extension of the writer's theory of the origin of the spurious mutants in *Oenothera* by means of the rare formation of new combinations between heterozygous visible genes (or groups of genes) and balanced lethals that were linked with them. The fact that the linkage is now found to be partly or wholly inter- instead of intra-chromosomal does not destroy the validity of this general mechanism. It means rather, as Darlington has pointed out, that the balanced lethal alignment is probably of positive value from its effect in enforcing heterozygosis on other loci besides those of these lethals themselves; and the translocations which caused the inter-chromosomal linkage tended to survive because they permitted an extension of this temporarily advantageous effect to previously disjoined portions of the chromatin. Even without a positive survival value, however, such translocations would tend to accumulate in a race already possessing balanced lethals, because they would not appreciably lower productivity so long as they determined the direction of chromosome segregation.

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THEORETICAL GENETICS OF AUTOPOLYPLOIDS.

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AN increasing amount of practical genetics is now being done on polyploid plants. These may be divided into allopolyploids, in which each chromosome has only one normal mate, although occasionally an abnormal pairing occurs, and autopolyploids in which a chromosome is equally likely to pair with any of two or more others. There appear to be intermediate types as well. At this institution researches are at present being carried out on three autopolyploids, namely the tetraploid forms of *Primula sinensis*, *Campanula persicifolia*, and *Lycopersicum esculentum*. The genetics of *Dahlia variabilis*, which behaves in some respects as an autopolyploid, are also being studied. The present paper deals with the genetical behaviour to be expected from such plants. Linkage is not considered, as this is being dealt with in a forthcoming paper by de Winton and Haldane on *Primula sinensis*. A gamete or zygote containing x of the factor A and y of the factor a is called $A^x a^y$. Thus $A^3 a$ denotes $AAAAa$, and so on. We shall only consider orthoploids, i.e. polyploids containing an even number of sets of chromosomes.

GAMETIC SERIES.

The gametic series to be expected from each class of zygote are given in Table I. The results for tetraploids and octaploids have already been given by Muller (1914) and Lawrence (1929). To save space the gametic output of homozygotes is omitted. Thus an A^{10} decaploid produces only A^5 gametes, an a^{10} only a^5 , and so on. After the hexaploid some of the gametic series are omitted. Thus a decaploid of constitution $A^2 a^8$ would give a gametic series $2A^2 a^3 : 5Aa^4 : 2a^5$, the same as that of $A^8 a^2$, with A and a interchanged.

The method of calculation is simple, as the following example shows. Consider a decaploid $A^7 a^3$. Five out of ten factors can be chosen in $\frac{10.9.8.7.6}{5.4.3.2.1}$ or 252 ways. Of these either an A^5 or $A^2 a^3$ gamete can be chosen in $\frac{7.6}{2.1}$ or 21 ways, an $A^4 a$ or $A^3 a^2$ in $\frac{7.6.5}{3.2.1} \times 3$, or 105 ways.

TABLE I.

$2m$	Zygote	Gametes
4	$A^3 a$ $A^2 a^2$ $A a^3$	$1A^3: 1A a$ $1A^2: 4A a: 1 a^2$ $1A a: 1 a^2$
6	$A^5 a$ $A^4 a^2$ $A^3 a^3$ $A^2 a^4$ $A a^5$	$1A^3: 1A^2 a$ $1A^3: 3A^2 a: 1A a^2$ $1A^3: 9A^2 a: 9A a^2: 1a^3$ $1A^2 a: 3A a^2: 1a^3$ $1A a^2: 1a^3$
8	$A^7 a$ $A^6 a^2$ $A^5 a^3$ $A^4 a^4$ etc.	$1A^4: 1A^3 a$ $3A^4: 8A^3 a: 3A^2 a^2$ $1A^4: 6A^3 a: 6A^2 a^2: 1A a^3$ $1A^4: 16A^3 a: 36A^2 a^2: 16A a^3: 1a^4$
10	$A^9 a$ $A^8 a^2$ $A^7 a^3$ $A^6 a^4$ $A^5 a^5$ etc.	$1A^5: 1A^4 a$ $2A^5: 5A^4 a: 2A^3 a^2$ $1A^5: 5A^4 a: 5A^3 a^2: 1A^2 a^3$ $1A^5: 10A^4 a: 20A^3 a^2: 10A^2 a^3: 1A^4 a$ $1A^5: 25A^4 a: 100A^3 a^2: 100A^2 a^3: 25A^4 a: 1a^5$
12	$A^{11} a$ $A^{10} a^2$ $A^9 a^3$ $A^8 a^4$ $A^7 a^5$ $A^6 a^6$ etc.	$1A^6: 1A^5 a$ $5A^6: 12A^5 a: 5A^4 a^2$ $2A^6: 9A^5 a: 9A^4 a^2: 2A^3 a^3$ $1A^6: 8A^5 a: 15A^4 a^2: 8A^3 a^3: 1A^2 a^4$ $1A^6: 15A^5 a: 50A^4 a^2: 50A^3 a^3: 15A^2 a^4: 1A a^5$ $1A^6: 36A^5 a: 225A^4 a^2: 400A^3 a^3: 225A^2 a^4: 36A a^5: 1a^6$
16	$A^{15} a$ $A^{14} a^2$ $A^{13} a^3$ $A^{12} a^4$ $A^{11} a^5$ $A^{10} a^6$ $A^9 a^7$ $A^8 a^8$ etc.	$1A^8: 1A^7 a$ $7A^8: 16A^7 a: 7A^6 a^2$ $1A^8: 4A^7 a: 4A^6 a^2: 1A^5 a^3$ $5A^8: 32A^7 a: 56A^6 a^2: 32A^5 a^3: 5A^4 a^4$ $1A^8: 10A^7 a: 28A^6 a^2: 28A^5 a^3: 10A^4 a^4: 1A^3 a^5$ $1A^8: 16A^7 a: 70A^6 a^2: 112A^5 a^3: 70A^4 a^4: 16A^3 a^5: 1A^2 a^6$ $1A^8: 28A^7 a: 196A^6 a^2: 490A^5 a^3: 490A^4 a^4: 196A^3 a^5: 28A^2 a^6: 1A a^7$ $1A^8: 64A^7 a: 784A^6 a^2: 3136A^5 a^3: 4900A^4 a^4: 3136A^3 a^5: 784A^2 a^6: 64A a^7: 1a^8$

Ratios of dominants to recessives when a given heterozygous type is crossed with a recessive (nulliplex):

Tetraploid	$A^2 a^2$	5:1; $A a^3$	1:1
Hexaploid	$A^3 a^3$	19:1; $A^2 a^4$	4:1; $A a^5$ 1:1
Octaploid	$A^4 a^4$	69:1; $A^3 a^5$	13:1; $A^2 a^6$ 11:3; $A a^7$ 1:1
Decaploid	$A^5 a^5$	251:1; $A^4 a^6$	41:1; $A^3 a^7$ 11:1; $A^2 a^8$ 7:2; $A a^9$ 1:1
Dodecaploid	$A^6 a^6$	923:1; $A^5 a^7$	131:1; $A^4 a^8$ 33:1; $A^3 a^9$ 10:1; $A^2 a^{10}$ 17:5; $A a^{11}$ 1:1

Heccaidecaploid $A^8 a^8$ 12,869:1; $A^7 a^9$ 1429:1; $A^6 a^{10}$ 285:1; $A^5 a^{11}$ 77:1; $A^4 a^{12}$ 25:1; $A^3 a^{13}$ 9:1; $A^2 a^{14}$ 23:7; $A a^{15}$ 1:1

The gametic series is therefore

$$21A^5: 105A^4a: 105A^3a^2: 21A^2a^3,$$

or

$$1A^5: 5A^4a: 5A^3a^2: 1A^2a^3.$$

In general from a zygote $A^r a^{2m-r}$, m factors can be chosen in $\frac{(2m)!}{(m!)^2}$

ways. A gamete $A^s a^{m-s}$ can be chosen in $\frac{r!}{(r-s)! s!} \cdot \frac{(2m-r)!}{(m-r+s)! (m-s)!}$ ways. Hence the probability of a zygote $A^r a^{2m-r}$ producing a gamete $A^s a^{m-s}$, is

$$\frac{(m!)^2 (2m-r)! r!}{(2m)! (m-s)! (m-r+s)! s! (r-s)!}$$

When $r = m$, i.e. the zygote is $A^m a^m$, this probability reduces to

$$\frac{(m!)^4}{(2m)! (m-s!)^2 (s!)^2}$$

RESULTS OF SELFING AND CROSSING.

The results of selfing any type can easily be calculated. For example, the result of selfing an octaploid of composition $A^3 a^5$ is given by the algebraic process of formally squaring the gametic series

$$1A^3a : 6A^2a^2 : 6Aa^3 : 1a^4,$$

i.e. by expanding $(A^3a + 6A^2a^2 + 6Aa^3 + a^4)^2$. The expected series is therefore

$$1A^6a^2 : 12A^5a^3 : 48A^4a^4 : 74A^3a^5 : 48A^2a^6 : 12Aa^7 : 1a^8,$$

i.e. one complete recessive, or nulliplex, in 196.

When two allelomorphic pure lines of a $2m$ -ploid are crossed, the result is a zygote $A^m a^m$. On selfing this we may expect one recessive in a number of zygotes equal to the square of the gametic series, i.e. one in $\frac{(2m!)^2}{(m!)^4}$, e.g. 1 in 4 when $m = 1$. The values of this number are given in Table II, up to $m = 16$.

TABLE II.

Diploid number $2m$	2	4	6	8	10	12	14	16
Number needed in F_2	4	36	400	4900	63,504	853,776	11,778,624	165,636,900

It is clear that in practical breeding work it would rarely be worth attempting to obtain pure recessive (nulliplex) plants in F_2 from anything higher than a hexaploid.

The results of crossing can easily be calculated by symbolic multiplication. Thus from $A^3 a^3 \times A^3 a^5$ we should expect a zygotic series corresponding to

$$(1A^4 + 6A^3a + 6A^2a^2 + 1Aa^3)(1A^3a + 6A^2a^2 + 6Aa^3 + 1a^4),$$

$$\text{i.e. } 1A^7a : 12A^6a^2 : 48A^5a^3 : 74A^4a^4 : 48A^3a^5 : 12A^2a^6 : 1Aa^7.$$

If selfing is carried on for several generations, homozygotes will appear in reasonable numbers, and in the long run the whole population will become homozygous. The final ratio of $A^{2m} : a^{2m}$ is the same as that of $A : a$ in the original population. We shall consider two cases where this ratio is unity.

When, in a tetraploid, A^4 and a^4 are crossed, F_1 is A^2a^2 . Let the zygotic series in F_n be:

$$p_n A^4 : q_n A^3a : r_n A^2a^2 : q_n Aa^3 : p_n a^4,$$

where

$$2p_n + 2q_n + r_n = 1.$$

Now the result of selfing A_3a is $1A^4 : 2A^3a : 1A^2a^2$, that of selfing A^2a^2 is $1A^4 : 8A^3a : 18A^2a^2 : 8Aa^3 : 1a^4$.

$$\therefore p_{n+1} = p_n + \frac{1}{4}q_n + \frac{1}{8}r_n,$$

$$q_{n+1} = \frac{1}{2}q_n + \frac{3}{8}r_n,$$

$$r_{n+1} = \frac{1}{2}q_n + \frac{1}{2}r_n.$$

Also $p_1 = q_1 = 0, r_1 = 1$.

$$\therefore 3q_{n+1} + 2r_{n+1} = \frac{5}{6}(3q_n + 2r_n).$$

$$\therefore 3q_n + 2r_n = 2\left(\frac{5}{6}\right)^{n-1},$$

$$3q_{n+1} - 2r_{n+1} = 6(3q_n - 2r_n).$$

$$\therefore 3q_n - 2r_n = -2\left(\frac{1}{6}\right)^{n-1}.$$

$$\begin{aligned} \therefore 2q_n + r_n &= \frac{7}{12}(3q_n + 2r_n) + \frac{1}{12}(3q_n - 2r_n) \\ &= \frac{7}{5} \cdot \left(\frac{5}{6}\right)^n - \left(\frac{1}{6}\right)^n. \end{aligned}$$

This is the proportion of heterozygotes of various kinds expected in F_n , i.e. after n generations of selfing. It is tabulated in Table III.

TABLE III.

n	1	2	3	4	5	6	∞
$2q_n + r_n$	1.0	.91	.805	.6744	.5625	.4685	0

In the corresponding case in the hexaploid the F_1 is A_3a_3 , the zygotic series in F_n :

$$p_n A^6 : q_n A^5a : r_n A^4a^2 : s_n A^3a^3 : r_n A^2a^4 : q_n Aa^5 : p_n a^6,$$

where $2p_n + 2q_n + 2r_n + s_n = 1$ and $p_1 = q_1 = r_1 = 0, s_1 = 1$.

$$\therefore p_{n+1} = p_n + \frac{1}{4}q_n + \frac{1}{25}r_n + \frac{1}{400}s_n,$$

$$q_{n+1} = \frac{1}{2}q_n + \frac{6}{25}r_n + \frac{9}{200}s_n,$$

$$r_{n+1} = \frac{1}{4}q_n + \frac{12}{25}r_n + \frac{99}{400}s_n,$$

$$s_{n+1} = \frac{12}{25}r_n + \frac{11}{100}s_n.$$

Solving the identity

$$q_{n+1} + \lambda r_{n+1} + \mu s_{n+1} \equiv k(q_n + \lambda r_n + \mu s_n),$$

we find $k = \frac{1}{20}, \frac{11}{25},$ or $\frac{9}{10}$.

$$\therefore 80q_{n+1} - 144r_{n+1} + 89s_{n+1} = \frac{1}{20}(80q_n - 144r_n + 89s_n) = \frac{1}{20}x_n,$$

$$25q_{n+1} - 6r_{n+1} - 12s_{n+1} = \frac{11}{25}(25q_n - 6r_n - 12s_n) = \frac{11}{25}y_n,$$

$$10q_{n+1} + 16r_{n+1} + 9s_{n+1} = \frac{9}{10}(10q_n + 16r_n + 9s_n) = \frac{9}{10}z_n.$$

$$\therefore x_n = 89\left(\frac{1}{20}\right)^{n-1}; y_n = -12\left(\frac{11}{25}\right)^{n-1}; z_n = 9\left(\frac{9}{10}\right)^{n-1},$$

$$1 - 2p_n = 2q_n + 2r_n + s_n$$

$$= \frac{23x_n + 1190y_n + 7007z_n}{50,830}$$

$$= \frac{4851}{3910}\left(\frac{9}{10}\right)^{n-1} - \frac{84}{299}\left(\frac{11}{25}\right)^{n-1} + \frac{89}{2210}\left(\frac{1}{20}\right)^{n-1}.$$

This expression gives the proportion of heterozygotes in F_n . After the first few generations it approximates to a geometric series whose successive terms are in the ratio 9:10, so the heterozygotes disappear quite slowly. Similar but more complicated expressions give the results of selfing in higher polyploids.

RANDOM MATING.

Some autopolyploids are self-fertile, even when the corresponding diploid is self-sterile. This is not, however, always the case. Thus Lawrence (1929) has shown that the self-sterile *Dahlia variabilis* behaves in some respects at least as an autotetraploid. The question therefore arises as to the results of random mating in a large population. Under any system of mating the ratio of dominant to recessive allelomorphs remains constant. This ratio will be called u . It is legitimate to regard the gametes of all plants as pooled in each generation, as this will obviously not affect the numbers of pairings of each type of gamete.

The following theorem holds good for all autopolyploids: "When equilibrium is reached under random mating of a $2m$ -ploid the gametes are produced in proportions given by the expansion of $(uA + 1a)^m$, the proportions of zygotic types being given by the expansion of $(uA + 1a)^{2m}$."

Thus in the case of a tetraploid the gametes will be in the ratios:

$$u^2AA : 2uAa : 1aa;$$

$$\text{the zygotes} \quad u^4A^4 : 4u^3A^3a : 6u^2A^2a^2 : 4uAa^3 : 1a^4.$$

In the case of a tetraploid it can readily be shown that such a population is in equilibrium. The general proof follows.

If the proportion of $A^s a^{m-s}$ gametes produced by one generation is

$$\frac{m! u^s}{(m-s)! s! (u+1)^m},$$

as follows from the above expansion, the proportion of $A^r a^{2m-r}$ zygotes is clearly

$$\frac{(2m)! u^r}{(2m-r)! r! (u+1)^{2m}}.$$

The probability of such a zygote producing an $A^s a^{m-s}$ gamete is

$$\frac{(m!)^2 (2m-r)! r!}{(2m)! (m-r+s)! (m-s)! (r-s)! s!},$$

as pointed out above. Hence the total proportion of $A^s a^{m-s}$ gametes produced by the next generation is

$$\begin{aligned} \sum_{r=0}^{2m} \frac{(2m)! u^r}{(2m-r)! r! (u+1)^{2m}} \cdot \frac{(m!)^2 (2m-r)! r!}{(2m)! (m-r+s)! (m-s)! (r-s)! s!} \\ = \frac{(m)! u^s}{(m-s)! s! (u+1)^m} \sum_{r=0}^{2m} \frac{(m)! u^{r-s}}{(m-r+s)! (r-s)! (u+1)^m} \\ = \frac{(m)! u^s}{(m-s)! s! (u+1)^m}. \end{aligned}$$

Hence the population is in equilibrium.

The following example shows how the above theory might be applied: "Three-quarters of a population of *Dahlia variabilis* have yellow flavone, i.e. possess the factor Y (Lawrence, 1929). If mating is at random, what proportions of the different genotypes may be expected?"

In a diploid we should expect $1YY:2Yy:1yy$. As Y shows tetraploid inheritance, $(u+1)^{-4} = \frac{1}{4}$, $\therefore u = \sqrt{2} - 1$. Hence we should expect to find:

$$\frac{(\sqrt{2}-1)^4}{4} Y^4 : (\sqrt{2}-1)^3 Y^3 y : \frac{3}{2} (\sqrt{2}-1)^2 Y^2 y^2 : (\sqrt{2}-1) Y y^3 : \frac{1}{4} y^4,$$

or 0.74 % Y^4 , 7.1 % $Y^3 y$, 25.74 % $Y^2 y^2$, 41.42 % $Y y^3$, 25.0 % y^4 .

It is significant that Lawrence, who analysed a number of individuals from a population where y^4 is a fairly common type, found no Y^4 , and only one $Y^3 y$.

THE RATE OF APPROACH TO EQUILIBRIUM.

Whereas a diploid population reaches equilibrium for an autosomal factor after one generation, this is not of course the case for a sex-linked factor, nor yet for an autosomal factor in a polyploid.

Consider a tetraploid population in which the n th generation produces (pooled) gametes in the proportions $x_n AA : 2y_n Aa : z_n aa$, where $x_n + 2y_n + z_n = 1$. Hence the $(n+1)$ th generation consists of:

$$x_n^2 A^4 : 4x_n y_n A^3 a : (4y_n^2 + 2x_n z_n) A^2 a^2 : 4y_n z_n A a^3 : z_n^2 a^4.$$

$$\therefore x_{n+1} = x_n^2 + 2x_n y_n + \frac{2}{3}y_n^2 + \frac{1}{3}x_n z_n = x_n + \frac{2}{3}(y_n^2 - x_n z_n),$$

$$y_{n+1} = x_n y_n + \frac{4}{3}y_n^2 + \frac{2}{3}x_n z_n + y_n z_n = y_n - \frac{2}{3}(y_n^2 - x_n z_n),$$

$$z_{n+1} = \frac{2}{3}y_n^2 + \frac{1}{3}x_n z_n + 2y_n z_n + z_n^2 = z_n + \frac{2}{3}(y_n^2 - x_n z_n).$$

Let $t_n = y_n^2 - x_n z_n$.

$$\begin{aligned}\therefore t_{n+1} &= (y_n - \frac{2}{3}t_n)^2 - (x_n + \frac{2}{3}t_n)(z_n + \frac{2}{3}t_n) \\ &= \frac{1}{3}t_n.\end{aligned}$$

$$\therefore t_n = 3^{-n}t_0.$$

If u be the ratio of $A : a$,

$$\therefore x_n + y_n = \frac{u}{u+1}, \quad y_n + z_n = \frac{1}{u+1}.$$

$$\therefore t_n = y_n^2 - \left(\frac{u}{u+1} - y_n\right)\left(\frac{1}{u+1} - y_n\right) = y_n - \frac{u}{(u+1)^2}.$$

$$\therefore x_n = \frac{u^2}{(u+1)^2} - 3^{-n}t_0,$$

$$y_n = \frac{u}{(u+1)^2} + 3^{-n}t_0,$$

$$z_n = \frac{1}{(u+1)^2} - 3^{-n}t_0.$$

Hence the approach to equilibrium is very rapid. Thus in the case of a population originally consisting only of homozygotes A^4 and a^4 , $u = 1$, $t_0 = -\frac{1}{4}$.

Hence the percentages of homozygotes in successive generations are 100, 50, 22.2, 15.43, 13.44, 12.81, 12.60, etc., the final value being 12.50.

In a hexaploid population let the gametic series produced by the n th generation be

$$p_n A^3 : 3q_n A^2 a : 3r_n A a^2 : s_n a^3,$$

where

$$p_n + 3q_n + 3r_n + s_n = 1,$$

so that
$$p_n + 2q_n + r_n = \frac{u}{u+1}, \quad q_n + 2r_n + s_n = \frac{1}{u+1}.$$

Then the zygotic series produced is:

$$p_n^2 A^6 : 6p_n q_n A^5 a : (9q_n^2 + 6p_n r_n) A^4 a^2 : (2p_n s_n + 18q_n r_n) A^3 a^3, \text{ etc.}$$

$$\begin{aligned}\therefore p_{n+1} &= p_n^2 + 3p_n q_n + \frac{1}{5}(9q_n^2 + 6p_n r_n) + \frac{1}{10}(p_n s_n + 9q_n r_n) \\ &= p_n + \frac{9}{10}(2q_n^2 - p_n r_n + q_n r_n - p_n s_n).\end{aligned}$$

$$q_{n+1} = q_n + \frac{3}{10}(4p_n r_n - 4q_n s_n + p_n s_n - q_n r_n + 2r_n^2 + 2q_n s_n), \text{ etc.}$$

On putting

$$x_n = q_n^2 - p_n r_n,$$

$$y_n = p_n s_n - q_n r_n,$$

$$z_n = r_n^2 - q_n s_n.$$

$$\therefore p_{n+1} = p_n + \frac{9}{10} (2x_n - y_n),$$

$$q_{n+1} = q_n + \frac{3}{10} (-4x_n + y_n + 2z_n),$$

$$r_{n+1} = r_n + \frac{3}{10} (2x_n + y_n - 4z_n),$$

$$s_{n+1} = s_n + \frac{9}{10} (-y_n + 2z_n).$$

Substituting

$$p_n = \frac{u}{u+1} - 2q_n - r_n, \quad s_n = \frac{1}{u+1} - q_n - 2r_n.$$

$$\therefore x_n = (q_n + r_n)^2 - \frac{ur_n}{u+1},$$

$$y_n = 2(q_n + r_n)^2 - \frac{2q_n + uq_n + r_n + 2ur_n}{u+1} + \frac{u}{(u+1)^2},$$

$$z_n = (q_n + r_n)^2 - \frac{q_n}{u+1}.$$

$$\therefore q_{n+1} = q_n + \frac{3}{10} \left[\frac{-4q_n - uq_n - r_n + 2ur_n}{u+1} + \frac{u}{(u+1)^2} \right],$$

$$r_{n+1} = r_n + \frac{3}{10} \left[\frac{2q_n - uq_n - r_n - 4ur_n}{u+1} + \frac{u}{(u+1)^2} \right].$$

Let

$$v_n = q_n + r_n, \quad w_n = q_n - r_n.$$

$$\therefore v_{n+1} = \frac{2}{5} v_n + \frac{3}{5} \frac{u}{(u+1)^2}.$$

$$\therefore v_n = \frac{u}{(u+1)^2} + \left(\frac{2}{5}\right)^n \left[v_0 - \frac{u}{(u+1)^2} \right],$$

$$w_{n+1} = \frac{1}{10} w_n + \frac{9(u-1)}{10(u+1)} v_n.$$

$$\therefore w_n = \frac{(1-10^{-n})u(u-1)}{(u+1)^3}$$

$$+ 10^{-n} w_0 + 3 \left[\left(\frac{2}{5}\right)^n - 10^{-n} \right] \frac{(u-1)}{(u+1)} \left[v_0 - \frac{u}{u+1} \right].$$

$$\therefore q_n = \frac{u^2}{(u+1)^3} + \frac{\left(\frac{2}{5}\right)^n (1-2u)}{u+1} \left[\frac{u}{(u+1)^2} - q_0 - r_0 \right] + \frac{10^{-n} u (u-1)}{(u+1)^3}$$

$$- \frac{10^{-n} [(u-2)q_0 + (2u-1)r_0]}{u+1}.$$

$$r_n = \frac{u}{(u+1)^3} + \frac{\left(\frac{2}{5}\right)^n (u-2)}{u+1} \left[\frac{u}{(u+1)^2} - q_0 - r_0 \right] - \frac{10^{-n} u (u-1)}{(u+1)^3}$$

$$+ \frac{10^{-n} [(u-2)q_0 + (2u-1)r_0]}{u+1}.$$

The corresponding expressions for p_n and s_n can readily be calculated. These numbers settle down to equilibrium only a little more slowly than in the tetraploid case, the ratio of successive differences from the final value being approximately $\frac{2}{3}$ instead of $\frac{1}{3}$. The higher polyploids approach equilibrium more slowly still.

EQUILIBRIUM IN A TETRAPLOID POPULATION WHICH IS
PARTLY SELF-FERTILISED.

Suppose that a proportion λ of each generation is formed by random mating, the remainder by self-fertilisation. Let u have the same meaning as before, and let the population in equilibrium consist of:

$$pA^4 : 4qA^3a : 6rA^2a^2 : 4sAa^3 : ta^4,$$

the pooled gametic series being $xA^2 : 2yAa : za^2$, where

$$x = p + 2q + r, \quad y = q + 2r + s, \quad z = r + 2s + t.$$

Let
$$p + 4q + 6r + 4s + t = x + 2y + z = 1,$$

$$\therefore x + y = \frac{u}{1+u}, \quad y + z = \frac{1}{1+u}.$$

Then, since all these quantities are unchanged from one generation to another:

$$p = (1 - \lambda) \left(p + q + \frac{1}{6}r \right) + \lambda x^2,$$

$$q = (1 - \lambda) \left(\frac{1}{2}q + \frac{1}{3}r \right) + \lambda xy,$$

$$r = (1 - \lambda) \left(\frac{1}{6}q + \frac{1}{2}r + \frac{1}{6}s \right) + \lambda \left(\frac{2}{3}y^2 + \frac{1}{3}xz \right),$$

$$s = (1 - \lambda) \left(\frac{1}{3}r + \frac{1}{2}s \right) + \lambda yz,$$

$$t = (1 - \lambda) \left(\frac{1}{6}r + s + t \right) + \lambda z^2.$$

$$\therefore y = q + 2r + s = \frac{5(1-\lambda)}{6} (q + 2r + s) + \lambda (xy + \frac{4}{3}y^2 + \frac{2}{3}xz + yz).$$

$$\therefore (1 - \lambda) y = 4\lambda (xz - y^2) = 4\lambda \left[\frac{u}{(u+1)^2} - y \right].$$

$$\therefore y = \frac{4\lambda u}{(1 + 3\lambda)(u + 1)^2}.$$

$$\begin{aligned} 6r &= (1 - \lambda) (q + 3r + s) + 2\lambda (2y^2 + xz) \\ &= (1 - \lambda) (r + y) + 2\lambda \left[3y^2 + \frac{(1 - \lambda)y}{4\lambda} \right]. \end{aligned}$$

$$\therefore r = \frac{3y(1 - \lambda + 4\lambda y)}{2(5 + \lambda)}.$$

y can be eliminated from this expression, and by somewhat tedious but quite straightforward algebra we arrive at the equations:

$$\begin{aligned}
 p &= \frac{u}{u+1} - \frac{4\lambda u}{(1+3\lambda)(u+1)^2} \left[1 + \frac{(1-\lambda)(7-\lambda)}{2(1+\lambda)(5+\lambda)} \right. \\
 &\quad \left. + \frac{4\lambda u}{(1+\lambda)(u+1)} - \frac{24\lambda^2 u}{(1+3\lambda)(5+\lambda)(u+1)^2} \right], \\
 q &= \frac{4\lambda u}{(1+3\lambda)(u+1)^2} \left[\frac{(1-\lambda)^2}{(1+\lambda)(5+\lambda)} \right. \\
 &\quad \left. + \frac{2\lambda u}{(1+\lambda)(u+1)} - \frac{24\lambda^2 u}{(1+3\lambda)(5+\lambda)(u+1)^2} \right], \\
 r &= \frac{6\lambda u}{(1+3\lambda)(5+\lambda)(u+1)^2} \left[1 - \lambda + \frac{16\lambda^2 u}{(1+3\lambda)(u+1)^2} \right], \\
 s &= \frac{4\lambda u}{(1+3\lambda)(u+1)^2} \left[\frac{(1-\lambda)^2}{(1+\lambda)(5+\lambda)} \right. \\
 &\quad \left. + \frac{2\lambda}{(1+\lambda)(u+1)} - \frac{24\lambda^2 u}{(1+3\lambda)(5+\lambda)(u+1)^2} \right], \\
 t &= \frac{1}{u+1} - \frac{4\lambda u}{(1+3\lambda)(u+1)^2} \left[1 + \frac{(1-\lambda)(7-\lambda)}{2(1+\lambda)(5+\lambda)} \right. \\
 &\quad \left. + \frac{4\lambda}{(1+\lambda)(u+1)} - \frac{24\lambda^2 u}{(1+3\lambda)(5+\lambda)(u+1)^2} \right].
 \end{aligned}$$

Each of these quantities thus varies continuously between the values corresponding to inbreeding and random mating as λ varies between 0 and 1. The proportion t of recessives is in general little affected by a small amount of random mating in a self-fertilised population, but a good deal by a small amount of self-fertilisation in a random mating population. Thus putting $u = 9$, 10 per cent. of self-fertilisation increases the proportion of recessives from 0.01 to 0.12 per cent., while 10 per cent. of random mating only diminishes it from 10 to 5.5 per cent. Similar calculations could be made from higher polyploids, but would be very tedious except in the symmetrical case $u = 1$.

CASES INVOLVING SEVERAL FACTORS.

All these cases can be generalised so as to apply to organisms heterozygous for several factors. Thus the gametic series produced by a plant of composition $A^4a^4B^2b^6$ is the expansion of

$$(1A^4 + 16A^3a + 36A^2a^2 + 16Aa^3 + 1a^4)(3B^2b^2 + 8Bb^3 + 3b^4),$$

a series of 15 terms. If dominance were complete, the series would be

$$759AB : 207Ab : 11aB : 3ab.$$

The results in F_2 from homozygous lines differing in two unlinked dominant factors are 9:3:3:1 in a diploid; 1225:35:35:1 in a tetraploid; 159,201:399:399:1 in a hexaploid, and so on. In practice one would not, even in a tetraploid, attempt to obtain a^4b^4 in the F_2 from $A^4b^4 \times a^4B^4$, but self a^4 and b^4 individuals in F_2 , most of which would give double recessives in F_2 .

The most striking consideration arising from an extension of the calculations on selfing to m factors is the extreme difficulty of establishing a homozygous dominant line by mere selfing. This is fully borne out by experience in *Primula sinensis*. Thus in F_6 , i.e. after five generations of selfing an F_1 , Table III shows that in a tetraploid there would be 26.6 per cent. of homozygous (quadriplex) dominants for a single factor, 46.85 per cent. of various heterozygotes, and 26.6 per cent. of recessives. Thus 36.2 per cent. of the dominants would be homozygous. But if the original population had been heterozygous for three unlinked factors only 0.362³, or 4.71 per cent. of the triple dominants would be homozygous for all three factors. In the corresponding case in a diploid 93.9 per cent. of single dominants would be homozygous for one factor, and 82.9 per cent. of the triple dominants homozygous for all three. Thus the probability of establishing a pure line in a self-fertile tetraploid is very small. In a self-sterile tetraploid or a higher polyploid it is negligible.

The populations reached as the result of random mating can be calculated with ease. Thus if u is the ratio of $A : a$, v that of $B : b$ in the population, the population in equilibrium is given by the expansion of $(uA + 1a)^{2m} (vB + 1b)^{2m}$. The approach to equilibrium remains fairly rapid in a tetraploid even when several factors are involved.

DOUBLE REDUCTION.

In a diploid organism equational non-disjunction, leading to the presence in one gamete of two chromosomes descended from the same single zygotic chromosome, implies an abnormal chromosome complement in the gamete. This is not so in a polyploid. Before meiosis each chromosome of a set has split into two chromatids, and if both the subsequent divisions are reductional, as opposed to equational, it is theoretically possible for both the chromosomes in a gamete to be derived from a pair of chromatids derived from one chromosome.

It is easy to calculate what should happen if the eight chromatids of a tetraploid are distributed at random into the gametes. A zygote $Aaaa$ gives eight chromatids $AAaaaaaa$. There are 28 combinations of these,

two at a time, namely $1AA : 12Aa : 15aa$, so this is the gametic series to be expected. Similarly $AAaa$ should give $3AA : 8Aa : 3aa$. In general the probability of obtaining from a zygote $A_r a_{2m-r}$ a gamete $A_s a_{m-s}$ is

$$\frac{3m! m! 2r! (4m - 2r)!}{4m! S! (2r - s)! (m - s)! (3m - 2r + s)!}$$

When $Aaaa$ is crossed with $aaaa$ we should obtain a zygotic ratio $13A : 15a$, as compared with $1A : 1a$ on the simple theory, while $AAaa$ would give $11A : 3a$, as compared with $5A : 1a$. The most striking differences are that 1 in 13 of the A zygotes from $Aaaa \times aaaa$ should be of composition $AAaa$, and that $AAAa \times aaaa$ should give 1 recessive to 27 dominants.

In *Primula sinensis* neither of these phenomena have been observed, and the ratios obtained agree pretty well with Muller's theory. In *Datura stramonium* Blakeslee, Belling and Farnham dealt with two unlinked factors P and A . P gave results agreeing very well with Muller's theory. A diverged in the direction here indicated, the figures obtained being intermediate between those expected on Muller's theory and on a basis of random assortment of chromatids.

On the latter theory the numbers in the second row of Table VI would be successive values of $\left(\frac{4m! m!}{3m! 2m!}\right)^2$, and the first five would be 4, 21.7, 121, 676, and 3785.2, so nulliplex individuals would be much commoner in F_2 than on Muller's theory.

The proportion of heterozygotes in tetraploid F_n would be

$$\frac{77}{65} \left(\frac{11}{14}\right)^{n-1} - \frac{12}{65} \left(\frac{6}{14}\right)^{n-1},$$

giving an ultimately rather faster rate of decrease than in Table III.

The stable population under random mating gives a pooled gametic series:

$$(5u^2 + u) AA : 8uAa : (u + 5) aa,$$

so that the proportion of nulliplex zygotes is $\frac{(1 + u/5)^2}{(1 + u)^4}$, the zygotic series is $9A^4 : 24A^3a : 34A^2a^2 : 24Aa^3 : 9a^4$.

In cases where Muller's type of segregation is not followed, the true values doubtless lie somewhere between these and the values given elsewhere in this paper. However, in most cases so far known the latter seem to be nearly correct.

SEX-LINKAGE OF THE *HUMULUS* TYPE.

Winge (1929) has reported that in *Humulus japonicus* the female is of composition *XX*, the male *XXX*. It is worth considering the probable mode of inheritance of a sex-linked factor in this case, which borders on polyploidy. There are two possible types of heterozygous male. *AAa* males should give $1AA:2Aa$ male-producing, and $2A:1a$ female producing pollen-grains. *Aaa* males should give $2Aa:1aa$, and $1A:2a$. The expected offspring from the 12 different types of mating are given in Table IV. If *A* is dominant the possible ratios among females are $5:1$, $2:1$, $1:1$ and $1:2$; among males $5:1$, $2:1$ and $1:1$. In a population in equilibrium under random mating with a factorial ratio of $uA:1a$ it can easily be verified that the population is in equilibrium if it consists of:

females, $u^2AA:2uAa:1aa$; and males, $u^3AAA:3u^2AAa:3uAaa:1aaa$.

Hence the proportion of recessive females is $(u+1)^{-2}$, of recessive males $(u+1)^{-3}$, the latter being thus the $\frac{2}{3}$ power of the former, instead of the square, as with normal sex-linkage. The equilibrium is not reached at once. The rate of approach and the effects of inbreeding may easily be calculated as in the former cases. The departure from equilibrium is roughly reduced by $\frac{1}{3}$ in each generation.

TABLE IV.

Parents		Offspring	
♀	♂	♀	♂
<i>AA</i> × <i>AAA</i>		<i>AA</i>	<i>AAA</i>
<i>AA</i> × <i>AAa</i>		$2AA:1Aa$	$1AAA:2AAa$
<i>AA</i> × <i>Aaa</i>		$1AA:2Aa$	$2AAa:1Aaa$
<i>AA</i> × <i>aaa</i>		<i>Aa</i>	<i>Aaa</i>
<i>Aa</i> × <i>AAA</i>		$1AA:1Aa$	$1AAA:1AAa$
<i>Aa</i> × <i>AAa</i>		$2AA:3Aa:1aa$	$1AAA:3AAa:2Aaa$
<i>Aa</i> × <i>Aaa</i>		$1AA:3Aa:2aa$	$2AAa:3Aaa:1aaa$
<i>Aa</i> × <i>aaa</i>		$1Aa:1aa$	$1Aaa:1aaa$
<i>aa</i> × <i>AAA</i>		<i>Aa</i>	<i>Aaa</i>
<i>aa</i> × <i>AAa</i>		$2Aa:1aa$	$1AAa:2Aaa$
<i>aa</i> × <i>Aaa</i>		$1Aa:2aa$	$2Aaa:1aaa$
<i>aa</i> × <i>aaa</i>		<i>aa</i>	<i>aaa</i>

SUMMARY.

The gametic series to be expected from various types of heterozygous autopolyploids are given, and the effects of self-fertilisation and random mating on populations are considered.

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EXPERIMENTS ON THE GENETICS OF WILD POPULATIONS.

II. *PHELEUM PRATENSE* L. AND THE HYBRID *P. PRATENSE* L. \times *P. ALPINUM* L.

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(With Two Plates, One Text-figure and One Folding-figure.)

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I. EXAMINATION OF LIVING MATERIAL.

(By J. W. GREGOR.)

INTRODUCTION.

THE influence of man, as has been pointed out by Turesson (1929), is especially evident in the group of species which constitute the herbage plants. Directly or indirectly, many natural habitats have been altered by human activities, with the result that the plant communities and even the composition of species-populations have been changed. Moreover, the distribution of many plants has been in some cases greatly extended and in others curtailed by the accidental or deliberate action of man.

The present distribution of the species *Phleum pratense* L., commonly known as Timothy, offers an example of man's interference with nature. *P. pratense* is a native of the Old World, occurring in Europe and temperate Asia and extending from the Mediterranean northward to about lat. 70°. Although this species has long been extensively cultivated in America, the botanical evidence is strongly against its being indigenous to the New World. *P. alpinum* L., on the other hand, is not confined to the Old World but occurs in North America, is present in Iceland and Greenland, and has been recorded from Patagonia. Piper (1920) writes: "Most northern plants common to the Old and the New World range in North America either from Alaska southward through the Western mountains, or south-eastward to New England, or else from Greenland south to New England. It has been thought by some that Timothy (*P. pratense*) was native in New England, but as the plant is not native to the northward of New England, nor in Alaska and the Rocky Mountains, it is quite certain that the plant is not endemic to North America." *P. pratense*, therefore, was probably introduced into America during the early colonial period. It is, however, believed that Timothy was first brought into cultivation in the United States, and that it was from there that the cultivated Timothy was imported into Britain. According to Witte (1915), however, Timothy had already been cultivated in Sweden, under the name Ångskampe, prior to the introduction of seed from America. The species is widely distributed in Britain, and may be regarded as one of the important agricultural grasses.

COLLECTION AND CLASSIFICATION OF MATERIAL.

For the present investigation, in order to obtain representative types, collections of seed were made in Scotland and England: (1) from populations occupying natural or semi-natural habitats, some of these being closely grazed pastures while others were comparatively rough, (2) from waste places, and (3) from stocks of various cultivated Timothy strains from Scotland and other countries.

At the Scottish Plant Breeding Station all the populations raised from these samples received similar treatment. The seeds were sown in a greenhouse in January, and the seedlings were transplanted at an early stage of growth into boxes, whence they were transferred to the experimental field; they were spaced 45 cm. apart in rows at 75 cm. intervals. The populations were examined during both the first and second years of their growth.

In the following descriptions reference will be made to the measure-

ments of panicle height and panicle spread. The former is the vertical distance from the ground surface to the apex of the tallest panicle, and the latter is the maximum distance, through the centre of the plant, between the apices of the opposite marginal panicles.

From the study of the various populations it became apparent that the species *P. pratense* in Britain contained two distinct groups: (1) the "American" or "Cultivated," the term being here applied to types which were constantly found in the agricultural samples from America and Europe; (2) the British "Wild." Each group contains various growth-forms.

Group I. "American." This group is characterised by the development of comparatively few tillers, even in the second summer. The fertile stems are strong, and haplocorms¹ (Evans, 1927), consisting of one, two, or very occasionally three swollen internodes, are usually developed; haplocorms may not be present on all stems of a plant. There are approximately seven elongated internodes in the haplocorm and culm. The tillers rarely arise from nodes other than those of, or below, the haplocorm, or the one immediately above. The leaves are generally long and broad. This group is seldom, if ever, common in old closely grazed pastures.

Group II. British "Wild." This is a multi-tillering low-growing group as compared with Group I. The erect forms in culture seldom exceed 0.7 m. in height though, under similar conditions, the corresponding growth-form of Group I may attain a height of 1.4 m. The fertile stems are slender and haplocorms are often poorly developed, while in some plants they are completely absent. The number of elongated internodes per stem is similar to that for Group I. In prostrate and decumbent forms rooting tillers may arise from nodes up to, and including, the fourth; in a few rare instances rooting tillers were observed at the fifth nodes (the node immediately above the first elongated internode was counted as the first), but such plants cannot be regarded as truly stoloniferous. This group is also distinguishable by a smaller seed size than that of Group I. Matured seeds were collected in the greenhouse from several erect plants of both groups and the number per gram was counted; 1433 seeds of Group I were equivalent in weight to 2273 seeds of Group II. Leaf length and breadth were variable. In general this group is suited to grazing conditions.

¹ *Haplocorm* (Evans, 1927). The swollen part near the base of the stem immediately above the *proaxis* which is the part of the stem below the first elongated internode.

Radical leaves. Leaves arising from the *proaxis*.

Stem leaves. Leaves arising from the elongated portion of the stem.

GENETIC RELATIONSHIP BETWEEN GROUPS.

Reciprocal crosses were attempted between the two groups, and failure has followed all inter-group matings. Since hand-emasculations invariably resulted in the premature withering of the panicles, advantage was taken of the self-sterility which occurred in certain individual plants; clones known to be self-sterile or only slightly self-fertile, but of proved fertility in compatible combinations, were employed as female parents. No seed was obtained.

In order to differentiate further between the two *P. pratense* groups, crosses were made between members of each group and the species *P. alpinum*. No difficulty was experienced in obtaining hybrids from Group II *P. pratense* \times Scottish *P. alpinum* (identification mark *CbA* 11); moreover, natural crosses between self-sterile clones of Group II and *P. alpinum* were found to occur in the experimental field. On the other hand, many thousands of flowers of Group I were pollinated with Scottish *P. alpinum* but only a single hybrid plant resulted.

P. pratense (Group II) \times *P. alpinum* (Scottish). Approximately 600 F_1 plants have been studied, all of which were early flowering like the *P. alpinum* parents; their floral characters, also, bore a closer resemblance to those of the Scottish *P. alpinum*. The ratio total length of barren-glume/length of barren-glume point has been calculated for the parents and their hybrid, and the figures, which include those for Group I *P. pratense*, are given in Table I.

TABLE I.

Ratio Total length of barren-glume/Length of barren-glume point.

Type	No. of plants	Range		Mean	Coefficient of variability
		Min.	Max.		
Group I	116	2.5	5.5	3.6 \pm 0.08*	24.7 \pm 1.72
Group II	116	2.5	7.0	5.6 \pm 0.12	22.7 \pm 1.56
<i>CbA</i> 9 (F_1)	55	2.0	3.5	3.0 \pm 0.05	11.7 \pm 1.13
<i>P. alpinum</i>	570	1.5	5.0	2.4 \pm 0.02	17.5 \pm 0.54

* Standard error.

All the hybrids (identification mark *CbA* 9) were apparently intra-sterile, and every attempt to self- and cross-fertilise plants under control in the greenhouse failed. Nevertheless, approximately 500,000 unprotected flowers from F_1 plants (*CbA* 9) growing in the experimental field yielded 46 "seeds," only four of which germinated, giving rise to the plants numbered *CbA* 21 (1), (2), (3) and (4). The fertility of these four

plants was as follows: (1) male and female sterile, (2) some normal pollen-grains produced, female sterile, (3) male sterile, comparatively female fertile, (4) pollen production equal to *P. pratense* and *P. alpinum*, comparatively female fertile.

F_1 progeny (*CbA* 9) were also back-crossed to the Scottish *P. alpinum* (*CbA* 11). Many panicles of *CbA* 9 plants were pollinated but only five hybrids were obtained (*CbA* 22, *CbA* 23 (1) and (2), *CbA* 24 (1) and *CbA* 24 (a)), all of which were both male and female sterile.

It is important to differentiate between the Scottish *P. alpinum*, as represented by *CbA* 11, and certain other European forms of this species. A Swedish population (*CbA* 30), which was raised from seed kindly sent to us by Dr Fredrik Nilsson, Landskrona, is decidedly less vigorous in culture than the populations of Scottish origin, the mean weights of the plants in ounces for the Swedish and Scottish populations being respectively 6.0 ± 0.47 and 20.9 ± 0.65 . Plants of these two types when examined cytologically, as described later, were found to possess a chromosome difference.

P. pratense (Group I) \times *P. alpinum* (Scottish). As previously mentioned, this cross gave only one hybrid (*CbA* 18 VIII (1)), which in general character closely resembled the *CbA* 9 series but was considerably taller. All attempts to obtain seed have failed, and the plant also proved to be male sterile.

The failure to obtain hybrids from the cross Group I \times Group II, combined with the difference in behaviour between Group I \times *P. alpinum* and Group II \times *P. alpinum*, confirms the phenotypic classification of *P. pratense* into two groups. The cytological evidence is also of importance in this connection.

PHENOTYPIC CLASSIFICATION OF GROWTH-FORMS WITHIN THE SPECIES *P. PRATENSE* AT THE TIME OF FLOWERING.

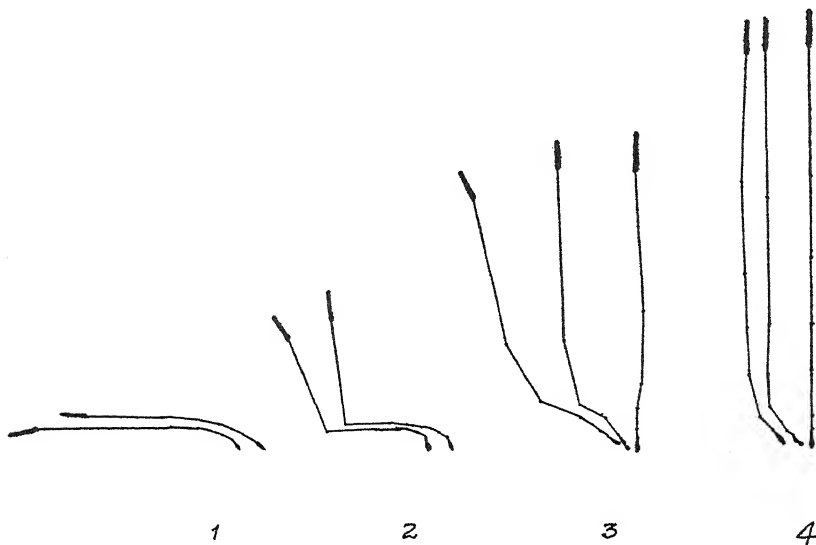
For purposes of classification four growth-forms may be distinguished, although there is no sharp line of demarcation between any one form and the next in the series.

1. *Prostrate*. Stems prostrate, panicles lying on, or only slightly raised above, the ground. This form has been observed solely in Group II (Text-fig. 1 (1), Plate XV, fig. 3).

2. *Decumbent*. Stems decumbent. In extreme forms, Text-fig. 1 (2), Plate XV, fig. 4, only distal internodes erect or ascending, panicles confined to periphery; in other members of this class stems kneed in an upward direction at more than one node, panicle distribution more

general but erect central stems absent. Ratio panicle height/panicle spread less than unity. Group I: Text-fig. 1 (2), Plate XV, fig. 1. Group II: Text-fig. 1 (2), Plate XV, fig. 4.

3. *Ascending*. Peripheral stems decumbent-ascending, upper internodes assuming an inclined position. Central stems sharply ascending but not truly erect. Ratio panicle height/panicle spread in Group I exceeds unity (Text-fig. 1 (3)), in Group II rarely exceeds unity (Text-fig. 1 (3), Plate XVI, fig. 5).



Text-fig. 1. Growth-forms of *P. pratense* L. represented diagrammatically.

4. *Erect*. Stems perpendicular except for lower internodes of peripheral ones, which sharply ascend. Ratio panicle height/panicle spread in Group I much greater than unity (Text-fig. 1 (4), Plate XV, fig. 2), in Group II exceeds unity (Text-fig. 1 (4), Plate XVI, fig. 6).

Relationship between growth habits of stem and leaf.

Decumbent plants, especially those with short leaves, give a general impression of a relatively large number of radical leaves (leaves arising from the elongated portion of the stem have been termed "stem leaves"). This results from the nodes of such plants lying close to the ground level, and the only stem leaf which is at first sight apparent is the comparatively small upper leaf; the condition is most marked when the plants possess short upper leaf sheaths and relatively long upper internodes. On the

other hand, in the erect type, a high proportion of the stem leaves is elevated above the ground surface.

ECOLOGICAL SIGNIFICANCE OF GROWTH-FORMS
(ECOTYPES OF TURESSON).

From the examination of populations growing in culture at Corstorphine, it is apparent that the two groups of *P. pratense* are suited to different environmental conditions. The populations collected from old, heavily grazed pastures contained solely the "Wild" group, but, in collections from certain temporary pastures, only occasional plants of this group were present in association with the predominating "American" group; both groups have been found in waste-place habitats where the grazing factor was absent, but Group II seems to be better suited to dry conditions.

In one waste-place habitat, occupied by plants of Group II, a single much-dwarfed plant with well-developed haplocorms was found. At the time, this plant was identified as *P. pratense* var. *nodosum*, but, when grown in culture, it assumed the typical Group I erect growth habit and, on being selfed, yielded a population indistinguishable from the "American" group. The cytological evidence has proved that it belongs to this group.

From the work of Evans (1927) on cultivated Timothy in America and from the observations of the present writer, it seems that the varietal name *nodosum* is superfluous, and its employment apparently serves no useful purpose in the classification of the British material. Although Ascherson and Graebner (1899) use the name *nodosum*, they mention that, in general, this form appears to be better characterised by the limp low-growing stem and the narrow panicle than by the knotty swellings at the base of the stem. Jenkin (1925) restricts the use of the name *nodosum* to a variety which chiefly occurs on chalk. The chalk forms which have been examined in culture at Corstorphine belong to Group II, and although swollen internodes are commonly formed, the condition is not more pronounced than in the case of certain other forms within Group II, or in the "American" group.

On account of the fact that both groups are widely distributed in Britain it is difficult to determine whether the "American" group existed in this country prior to the introduction of seed from America, although its wide distribution may be due to man's activities. Sinclair (1869) figures a native form of agricultural value, *P. pratense* var. *majus*, which apparently resembles a type in Group I, and an agricultural

Timothy is also mentioned by Parnell (1842) as being a hard, coarse grass common throughout the whole of Britain.

The adaptation of type to environment is illustrated not only by the fact that both groups seldom occupy the same habitat, but also by the relative proportions of the types exhibited by a group in different habitats. Populations of Group II derived from seed from habitats where the herbage was rough were taller in culture than those cultivated from closely grazed pastures. Table II gives the measurements of panicle height, panicle spread, and the ratio panicle height/panicle spread for four populations. In this table *Cb* 125 represents a population collected from an uncultivated locality where the herbage was dense and practically ungrazed; on the other hand, *Cb* 110 was collected from a natural pasture which was grazed by sheep. *Cb* 115 is from a pasture (near the sea) where the herbage was roughly grazed by cattle. *Cb* 18 is of cultivated Scottish origin and is included in the table for comparison.

TABLE II.

Comparison of populations Cb 110, 115, 125 (Group II) and Cb 18 (Group I).

1st year plants measured at flowering time.

Population no.	No. of variates	Range		Mean	Coefficient of variability
		Min.	Max.		
<i>Cb</i> 110	166	8	20	14.0 \pm 0.18*	16.6 \pm 0.94
<i>Cb</i> 115	59	9	19	15.2 \pm 0.30	15.2 \pm 1.41
<i>Cb</i> 125	136	8	26	19.4 \pm 0.28	16.8 \pm 1.05
<i>Cb</i> 18	137	22	38	30.3 \pm 0.30	11.7 \pm 0.71
Panicle spread in inches.					
<i>Cb</i> 110	166	6	28	15.5 \pm 0.33	27.7 \pm 1.62
<i>Cb</i> 115	59	7	29	14.6 \pm 0.51	27.2 \pm 2.67
<i>Cb</i> 125	136	8	24	15.2 \pm 0.25	19.3 \pm 1.20
<i>Cb</i> 18	137	4	13	7.9 \pm 0.13	19.8 \pm 1.22
Ratio Panicle height/Panicle spread.					
<i>Cb</i> 110	157	0.4	2.9	1.1 \pm 0.03	40.0 \pm 2.58
<i>Cb</i> 115	59	0.4	2.1	1.2 \pm 0.05	34.2 \pm 3.49
<i>Cb</i> 125	136	0.4	2.9	1.3 \pm 0.03	29.2 \pm 1.91
<i>Cb</i> 18	137	2.4	7.0	4.0 \pm 0.06	19.0 \pm 1.17

* Standard error of mean.

It is believed that in nature the types which are unsuited to the prevailing environmental conditions of their habitat are gradually eliminated, and those that survive bear at least a phenotypic resemblance to each other. Although phenotypic uniformity is frequently attained by wild populations to a considerable degree, a genotypic uniformity must indeed

be rare; under the more severe eliminating influences of artificial selection, such wild populations can again be separated into different growth-habit groups, the individual plants of each group being more or less uniform in appearance. The effect of artificial selection within population *Cb* 110 (Table II) is illustrated in Table III. Decumbent plants and plants representing the mean growth-habit of this population were selected. As the degree of self-fertility of the plants was low, similar phenotypes of each selection were crossed. Two populations, *Cb* 131 and *Cb* 135, arising from the average type and the low-growing type respectively, were grown in culture for two years, and from Table III it is obvious that they differed in growth-habit.

TABLE III.

Comparison of populations Cb 131 and 135 plants, measured (inches) at flowering time.

Panicle height. 1st year plants.						
Population no.	No. of variates	Range		Mean	Difference between means	Coefficient of variability
		Min.	Max.			
<i>Cb</i> 131	104	7	18	12.9 ± 0.22*	1.2 ± 0.35	17.5 ± 1.24
<i>Cb</i> 135	120	5	20	11.7 ± 0.27		24.9 ± 1.68
Panicle spread. 1st year plants.						
<i>Cb</i> 131	103	8	19	13.3 ± 0.19	3.9 ± 0.30	15.0 ± 1.06
<i>Cb</i> 135	120	11	25	17.2 ± 0.23		15.0 ± 0.97
Ratio Panicle height/Panicle spread.						
<i>Cb</i> 131	103	0.4	2.1	1.1 ± 0.02	0.4 ± 0.03	23.6 ± 1.72
<i>Cb</i> 135	120	0.2	1.4	0.7 ± 0.02		30.0 ± 2.08
Panicle height. 2nd year plants.						
<i>Cb</i> 131	102	18	26	22.6 ± 0.17	6.0 ± 0.25	7.4 ± 0.52
<i>Cb</i> 135	118	13	24	16.6 ± 0.19		12.3 ± 0.81
Panicle spread. 2nd year plants.						
<i>Cb</i> 131	102	16	28	21.6 ± 0.21	6.9 ± 0.33	9.8 ± 0.69
<i>Cb</i> 135	118	22	36	28.5 ± 0.25		9.6 ± 0.63
Ratio Panicle height/Panicle spread.						
<i>Cb</i> 131	102	0.7	1.4	1.1 ± 0.01	0.5 ± 0.01	13.0 ± 0.93
<i>Cb</i> 135	118	0.4	0.9	0.6 ± 0.01		17.1 ± 1.14

* Standard error of mean.

Observations on *P. pratense* and other plant species indicate that the more extreme are the environmental conditions of a habitat, the greater is the phenotypic similarity of the inhabiting forms and the more specialised their type.

There seems, therefore, to be little doubt that the processes of genotypic elimination, *i.e.* the continual destruction of segregates unsuited to

the prevailing conditions of the natural habitat, are mainly responsible for the population differentiation observed when wild material is brought under controlled cultural conditions.

II. CYTOLOGICAL EXAMINATION.

(By F. W. SANSOME.)

CHROMOSOME NUMBERS.

The results of a preliminary cytological examination of representatives of the various species and hybrids of *Phleum* described in Part I will be given here. The morphology and behaviour of the chromosomes will be described in a later paper wherein the relationships of the various species dealt with will be considered.

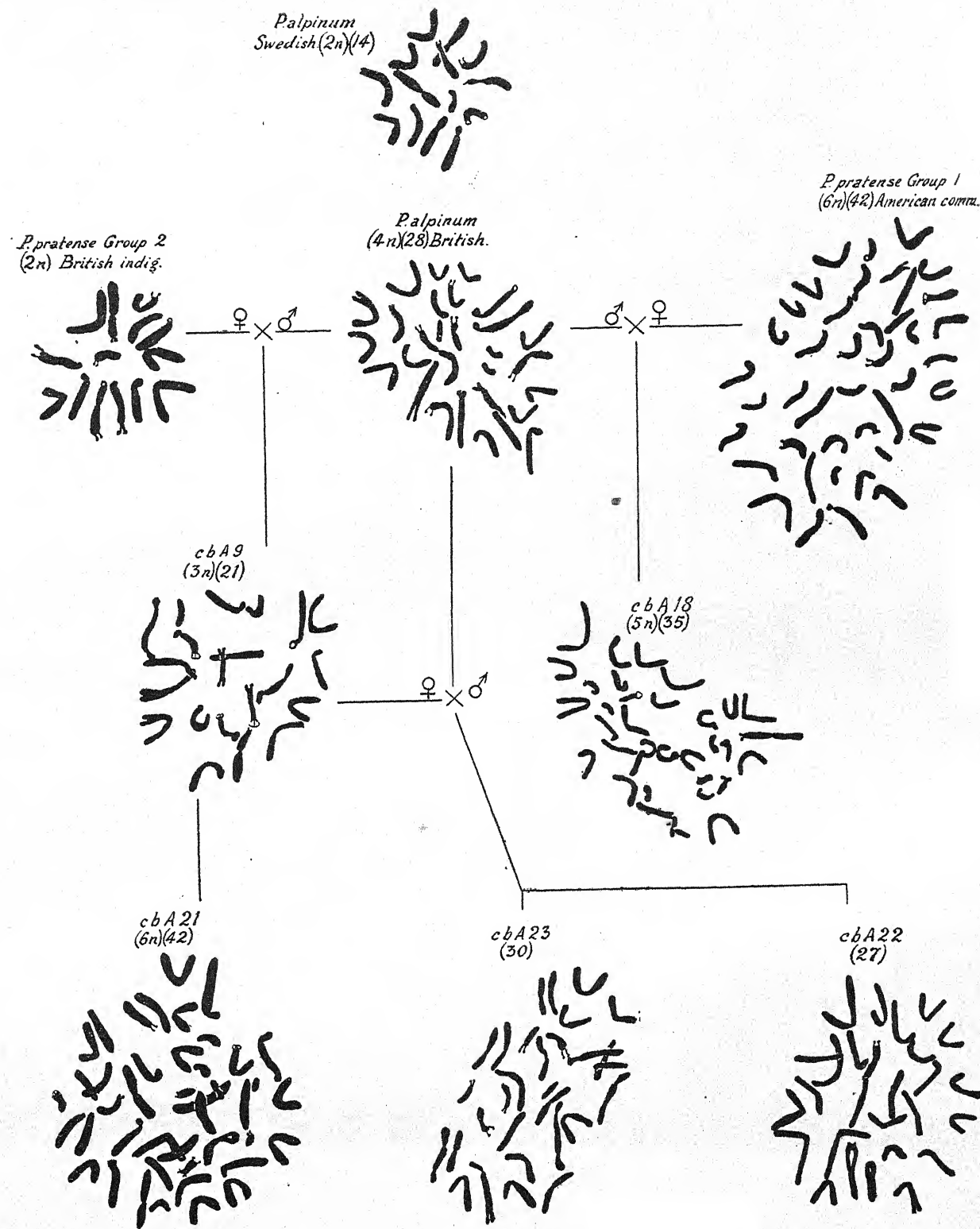
The fixative used was Flemming's medium fluid and the stain was gentian violet (Newton's method). The possibilities of the material were well demonstrated by the preparations of Mr La Cour of the John Innes Horticultural Institution, the constrictions and trabants on the chromosomes being well revealed. Table IV gives the somatic chromosome numbers of the plants described in the foregoing pages.

TABLE IV.

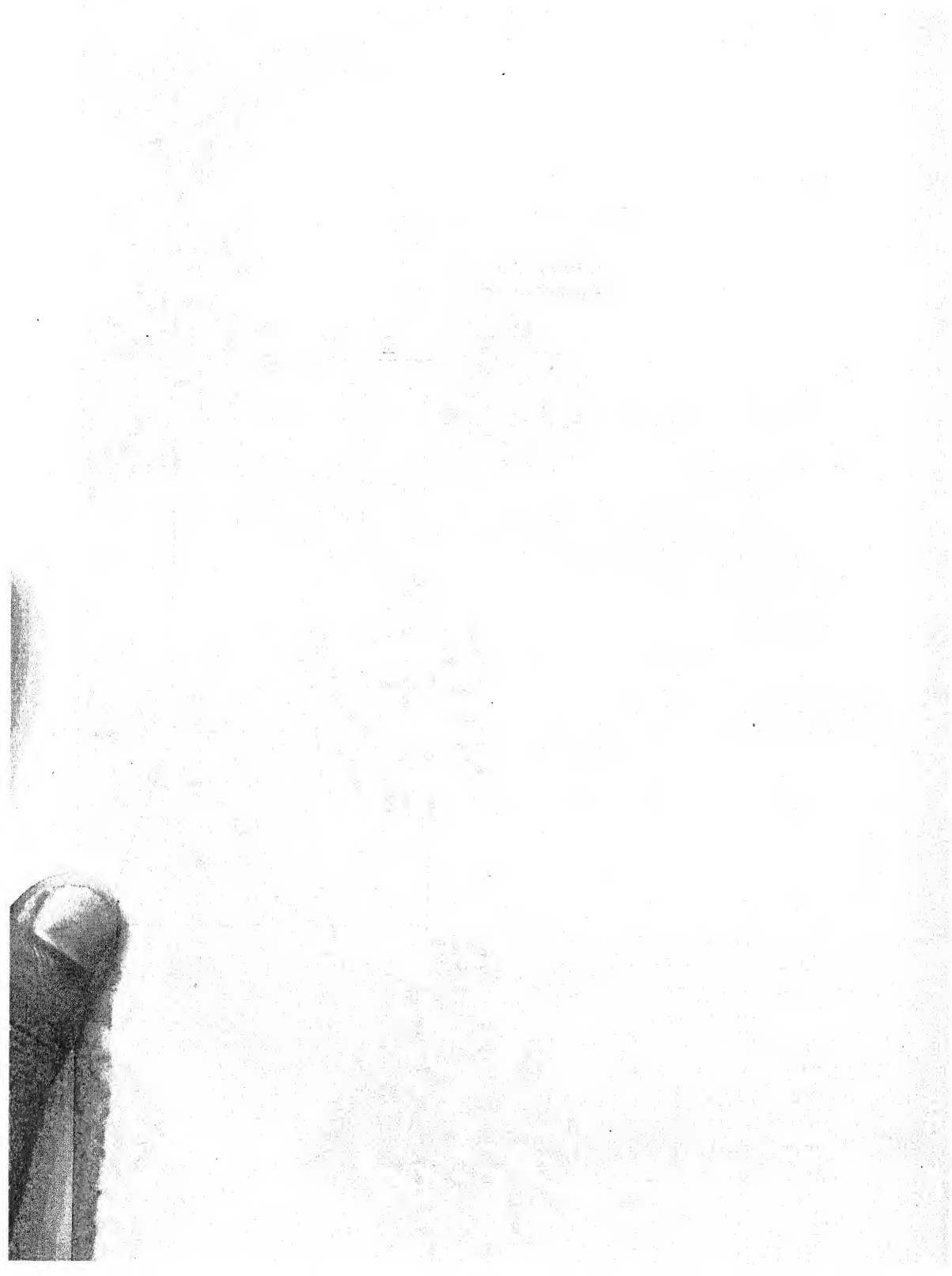
Station nos.	Description	Somatic chromosome nos.
Cb 94 (25)	<i>P. pratense</i> (Group I)	42 (6n)
Cb 99 (1)	" (Group II)	14 (2n)
CbA 30	<i>P. alpinum</i> (Sweden)	14 (2n)
CbA 11	" (Scotland)	28 (4n)
CbA 9	F_1 of <i>P. pratense</i> (Group II) \times <i>P. alpinum</i> (Scotland)	21 (3n)
CbA 21	F_2 of the above cross	42 (6n)
CbA 22	F_1 back-crossed to <i>P. alpinum</i> (Scotland)	27
CbA 23 (1)		26
CbA 23 (2)		30
CbA 18 VIII (1)	<i>P. pratense</i> (Group I) \times <i>P. alpinum</i> (Scotland)	35 (5n)

Seven is apparently the basic chromosome number of *Phleum*, as has been reported for a number of other genera of Gramineae (Huskins, 1927, Church, 1929, Faworski, 1927). The chromosome complements of the various crosses are illustrated in Text-fig. 2 (folding figure).

It will be observed that crosses were obtained between plants with different chromosome numbers. The cross *P. pratense* (2n) \times *P. alpinum* (4n) gave several plants all with a 3n number of chromosomes. These hybrids were partially sterile but gave rise to four plants, three of which were examined and found to be hexaploid. The triploid hybrid, however, proved more fertile when pollinated by *P. alpinum* (4n), and several



Text-fig. 2.



plants were obtained in which the chromosome numbers ranged from 26 to 30 (somatic).

The behaviour of this triploid F_1 is therefore analogous to several cases recently reported. It is probable that the hexaploids arose from the fusion of gametes having the somatic ($3n$) number of chromosomes. In *Hieracium* (Rosenberg, 1926), *Brassica* \times *Raphanus* (Karpechenko, 1927) and *Digitalis* (Buxton and Newton, 1928) the formation of restitution nuclei gives rise to gametes with the unreduced number of chromosomes. In such a triploid as the *Phleum* hybrid it is more probable that, in self-fertilisation, a zygote is formed by gametes arising from the formation of restitution nuclei, rather than by the normal process of reduction division which in a triploid is necessarily irregular.

The tetraploid *P. alpinum* parent presumably gave gametes with the $2n$ number of chromosomes, i.e. 14, so that, out of the 26 to 30 chromosomes found in the back-cross zygotes, 12 to 16 were probably furnished by the triploid hybrid parent.

The reduction division of a triploid plant will usually give rise to gametes with chromosome numbers ranging from n to $2n$, but occasionally gametes with chromosome numbers greater than $2n$ have been found (cf. Jørgensen, 1928). The question whether the production of gametes with 15 and 16 chromosomes by the *Phleum* triploid is due to non-disjunction, which necessarily implies homology among the chromosomes of the triploid, should be left until the pollen mother cells of the triploid have been examined. Darlington (1929) has shown that the production of viable gametes depends on (1) behaviour of the chromosomes at reduction division, (2) genetic balance of the resulting chromosome complement. In accordance with this, it is to be expected that a higher proportion of gametes with the higher chromosome numbers will be favoured in respect of viability.

The cross *P. pratense* (Group I ($6n$)) \times *P. alpinum* ($4n$) was obtained with difficulty. The one plant arising from this cross was completely sterile and was a pentaploid, the pollen mother cells of which contained univalents, bivalents and compound structures of higher valency at heterotypic division. Seed has probably been obtained from the cross artificial hexaploid \times *P. pratense* (Group I ($6n$)). The self-fertility of the artificial hexaploid parent is 0.5 per cent. and, although the cross-fertility of this mating is 5.4 per cent., it will be necessary to examine the progeny before certainty of a successful cross can be assumed.

The cytological facts above presented offer a satisfactory interpretation of the behaviour of *P. pratense* in breeding, and of its relationship

to *P. alpinum*. Without venturing on a systematic grouping of the several types, it may be mentioned that the cytological evidence indicates a common phylogeny for all the plants under observation and treated in this paper. It is interesting to find that *P. alpinum* can be crossed to both races of *P. pratense*, while these, up to the present, have not crossed with one another; this is to be expected on cytological grounds. If the $6n$ group of *P. pratense* are descendants of a species-cross analogous to the one we have studied, the fact that a tetraploid *P. pratense* has so far not been found may have considerable significance.

III. DISCUSSION AND CONCLUSIONS.

There are two apparently intersterile groups of *P. pratense*; of these Group I is essentially a hay type while Group II occurs chiefly in waste places and natural pastures, but both groups may sometimes be present in the same habitat. The cytological examination shows that Group I is probably hexaploid with 42 as the somatic chromosome number, while Group II is diploid (14). Within each group a relationship exists between the growth-forms of a particular habitat and the environmental conditions of that habitat. Both groups possess corresponding growth-forms, with the exception that in Group I the prostrate form has not been found. There is, however, a form in Group I which is more or less prostrate at the flowering stage but is definitely ascending during early growth. The different combinations of characters exhibited by plants of the same growth-habit make the total number of forms in the species very large. The authors are of the opinion that the survival of these growth-forms is dependent on their genotypic response to the environmental conditions of the habitats which they occupy.

The variability in *P. alpinum* of Scottish origin is considerably less than that of *P. pratense*. The distribution of *P. alpinum* in Britain is confined to a few isolated habitats on the Scottish mountains. This isolation of populations, in habitats where the selective influences of the environment are no doubt somewhat similar, may partly account for the lesser variability in the species. In this country the habitats of *P. alpinum* do not, to the writers' knowledge, overlap the areas occupied by *P. pratense* at any point, thus it is very improbable that natural crosses between the two species could occur at the present time. In the past, however, the distribution area of the two species may not have been thus separated, since even now on the European Continent, *P. alpinum* occasionally descends into the forest region where it meets *P. pratense* (Ascherson and Graebner (1899)). These authors state that, in the mountains, *P. pratense*

(sub-species *vulgare* A. and G.) attains an altitude of 1650 m.; according to Hegi (1906) the normal range of *P. alpinum* is from 1300 to 2400 m., although it sometimes reaches 2900 m., or grows as low as 1030 m. near Bludenz in Austria and 600 m. in Canton Glarus in East Switzerland.

As a result of cytological examination, two types of *P. alpinum* have been recognised: the Scottish type which is $4n$, and the Swedish material which, so far as examined, is $2n$. The tetraploid *P. alpinum* grows more vigorously in Scotland than diploid plants and would soon assume an ascendancy over the diploid species. Cold conditions are known to affect reduction division and it is possible that doubling of the somatic or gametic chromosomes may be induced by the environmental conditions of the habitat. It would not, therefore, be surprising to find tetraploid plants in populations of *P. alpinum* from other parts of the world.

It is tentatively suggested that *P. pratense* Group I ($6n$) may be the result of hybridisation of *P. pratense* Group II with some other plant, in a manner analogous to that described for the artificial hexaploid which resulted from the doubling of the chromosomes in gametes of the hybrid between *P. pratense* ($2n$) \times *P. alpinum* ($4n$). The relationships of the several strains mentioned in this paper will be more fully established when further data are available. At present, however, the authors prefer to regard *P. pratense* L. and *P. alpinum* L. as two distinct, though closely related, species rather than to adopt Ascherson's and Graebner's (1899) view that *P. pratense* has two sub-species, *P. vulgare* and *P. alpinum*. The geneecological data alone afford sufficient evidence of differences of specific rank between *P. pratense* and *P. alpinum*.

SUMMARY.

1. Two distinct, apparently intersterile, forms of *P. pratense* L. have been recognised and described as Group I and Group II; in general they occupy different ecological areas.

2. Corresponding hereditary growth-forms occur within each group; these also have ecological significance.

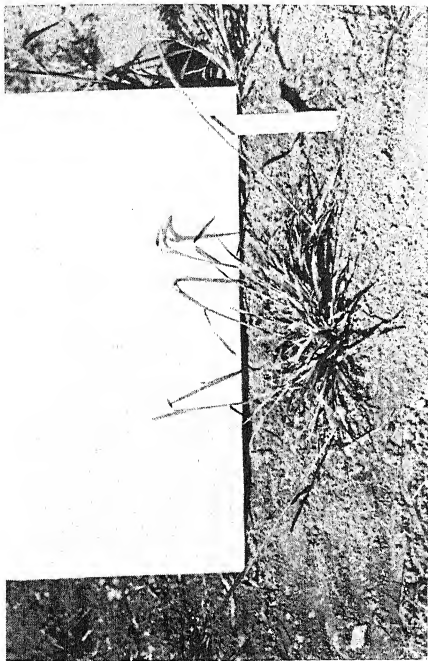
3. The phenotypical classification of *P. pratense* into two groups is in agreement with the cytological evidence.

4. Crosses between Group I ($6n$) and Group II ($2n$), and *P. alpinum* L. have been attempted with varying degrees of success. A hexaploid form has been obtained from the cross *P. pratense* ($2n$) \times *P. alpinum* ($4n$).

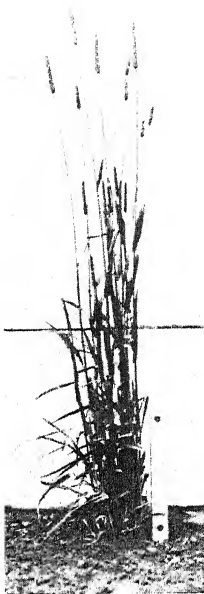
5. The basic chromosome number is seven. The chromosome complement of the various types has been determined as shown in Table IV.

LITERATURE.

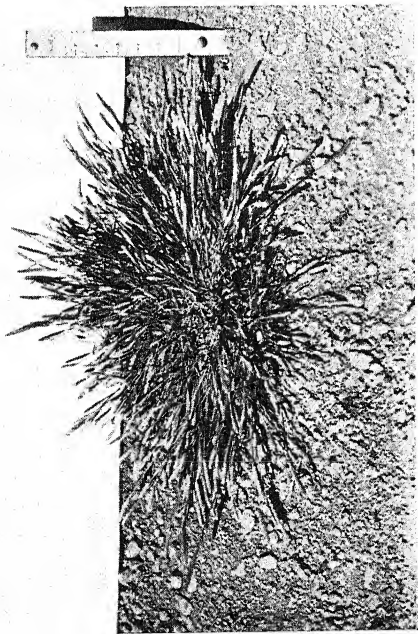
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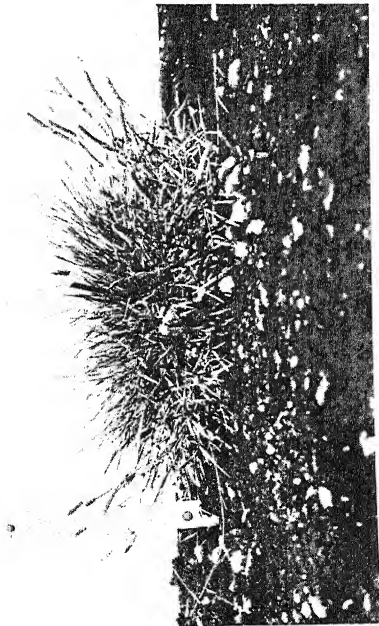
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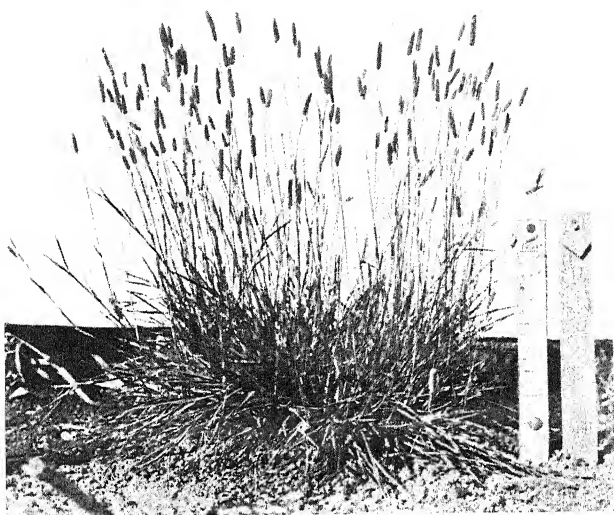
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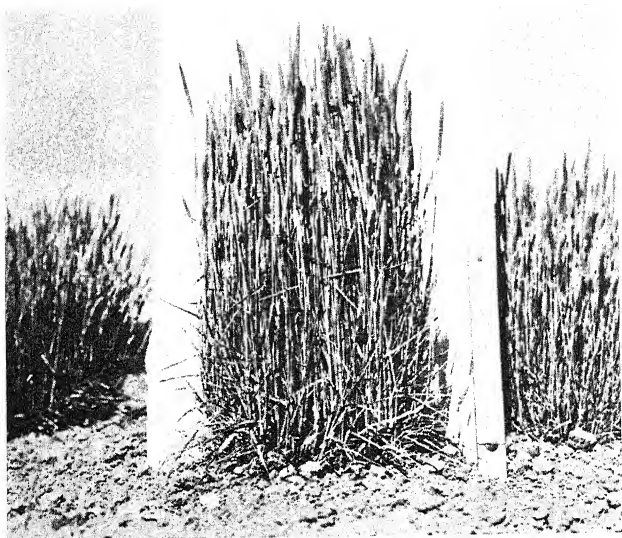
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EXPLANATION OF PLATES.

PLATE XV.

Phleum pratense L.

1. Group I. Decumbent form.
2. „ Erect form.
3. Group II. Prostrate form.
4. „ Decumbent form.

PLATE XVI.

Phleum pratense L.

5. Group II. Ascending form
6. „ Erect form.

INHERITANCE IN *LOLIUM PERENNE* L.

III. BASE-COLOUR FACTORS *C* AND *R*.

By T. J. JENKIN, M.Sc.

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INTRODUCTORY.

INDIVIDUAL plants of *Lolium perenne* vary in intensity of green colour from bright, somewhat yellowish green at the one extreme, to very dark green with a purplish tinge at the other. With very rare exceptions¹, however, naturally produced plants agree in that the lower part of the living sheath of the non-flowering tillers at or actually in the ground, is of a distinct red or purplish red, presumably owing to the development of anthocyanin pigment. This character is so constant that it has been generally accepted as one of the chief aids to the identification of the species in the absence of inflorescences(1, 4, 5).

Hessing(2) found that in *Lolium multiflorum* seedlings the coleoptiles might be either red or non-red. This is also true of *L. perenne*, but I have found that difference in coleoptile colour is insufficient as the basis of a classification of seedlings into red base and non-red since seedlings with apparently non-red coleoptiles may develop into red-base plants. It is even possible that the later growth stage adopted in the present instance, namely, that when the first secondary tiller has made some definite growth, is not sufficiently late for all coloured types, but it has been found entirely satisfactory in the case of the plants at present under discussion. In some cases, however, colour development is very small at this stage and can be most easily found at the base of the secondary tiller at a point to which very little, if any, light can penetrate. It would therefore seem that red base-colour is not dependent upon exposure to light, and this is supported by the fact that the colour is well developed in the centre tillers of the densest plants.

Seedlings which have been classified as non-red after reaching the growth stage mentioned have been found invariably to develop into non-red plants.

¹ The only exception of which I am aware is the strain mentioned by Watson(6). This strain, I am informed by Mr O'Brien, of the West of Scotland College of Agriculture, is derived from a few plants which he discovered in a field near Paisley.

The red base-colour is of the greatest value, for the identification of the species in the examination of grazed pastures, but it has not yet been determined whether it has any direct economic significance. In the course of the present work many thousands of seedlings have been observed, and, as a rule, the non-red appear to be somewhat less vigorous than the others. This may be due to the difference in colour alone, or it may be due to certain other factors which are linked to the colour factors. From the practical grass-breeder's point of view, this aspect of the question is of great importance, since in the grasses well-defined unit characters, which are not lethal or semi-lethal and can be used for the study of linkage relations, appear to be very rare.

METHODS AND MATERIAL.

In the present investigation the method of artificial hybridisation (hand-pollination after emasculation) has been extensively used¹. At the same time, all plants used as parents in the crosses have been self-pollinated, but owing to high self-sterility, poor germinating capacity in the seeds, or lack of vigour in the resulting seedlings, the results obtained by this method have rarely been quite satisfactory.

While studying single plants for economic purposes, two plants were found, on selfing, to give rise to both red base and non-red seedlings. Close in-breeding within each of the two lines so produced gave very poor results and consequently out-crosses were made to unrelated red base plants. Most of these out-crosses were made by hand, but three red base plants obtained by allowing non-red plants of Line 169 to produce seed under open pollination conditions have been used to some extent in further breeding work. For the study of the inheritance of red base-colour in each of the two lines, the plants bred within the lines or after out-crossing to unrelated plants have been used, while the two lines have also been interbred.

LINE 169.

One of the two original plants referred to above was given the reference number 169. This plant, from repeated self-pollination, gave red base and non-red seedlings in proportions strongly suggestive of a single factor difference. By in-breeding and out-crossing further heterozygous plants were produced. From selfing (26 families) and from inter-crossing (11 families) such plants produced a total of 1387 seedlings, of

¹ The method has elsewhere been described in detail. See Jenkins.

which 1040 were red base and 347 non-red. These figures agree as closely as possible with theoretical expectation on a 3 : 1 basis.

Heterozygous plants back-crossed to non-red gave red base and non-red seedlings in proportions showing fair agreement with expectation on a 1 : 1 basis, the actual figures being 312 and 331 respectively in the aggregate for 16 families.

Non-red plants, whether derived directly from Plant 169 by selfing or by extraction in the course of further breeding work, have given no red base progeny either when self-pollinated or when interbred (2198 seedlings examined).

No serious attempt has been made in the case of this line to identify plants homozygous for the red base character, but such plants have also been found.

In all instances the results for individual families within the groups, for which aggregate figures are given above, were in good general agreement with those for the group. It is therefore evident that the presence or absence of red base-colour in Line 169 is dependent upon a single factor difference, and the production of red colour may be considered to be due to the presence of the dominant factor C.

LINE 174.

Results obtained by selfing Plant 174 also very strongly suggested a unifactorial difference between red base and non-red seedlings. Germinating capacity in this case was, however, rather low, and generally in this line, while the individual plants were much more highly self-fertile than in Line 169, selfed seed germinated poorly. Many of the seedlings were also too weak to be classified. In crosses, particularly those made between in-bred and out-cross plants, poor germinating capacity was very rarely met with, while the seedlings were also vigorous.

Non-red plants, from selfing, gave a total of 1561 seedlings of which approximately 15 per cent. were too weak to be classified. All the others were of the non-red type.

Non-red plants in 15 crosses with plants heterozygous for base-colour gave a total of 1590 seedlings, 17 of which were too weak for classification. The remaining 1573 consisted of red base and non-red in the ratio 791 : 782.

One heterozygous red base plant, when selfed, gave a considerable, and apparently significant, excess of non-red seedlings as compared with expectation on a 3 : 1 basis, but when all the results obtained by selfing

and by intercrossing (9 crosses) heterozygous plants are aggregated the final result shows a deviation of 7 seedlings (excess of non-red) from expectation on this basis in a total of 3297 seedlings.

Five red base plants out of 12, obtained by out-crossing the parent plant 174 to unrelated red base plants, when back-crossed to non-red of the same line and/or to plants known to be heterozygous, gave only red base progeny (12 crosses: 1772 classifiable seedlings).

The results therefore definitely show that in Line 174, as in Line 169, red base-colour behaves as a simple Mendelian unit, although in rare cases somewhat abnormal ratios were obtained.

LINES 169 AND 174 INTERBRED.

Owing to the fact that only one non-red plant, and that a weak one, was then available, red base plants of Line 174, known to be heterozygous for base-colour, were largely used in the initial crosses. With these, both heterozygous red base and homozygous non-red plants of Line 169 were inter-crossed. In the former case, were the factor concerned with red base-colour identical for the two lines, red base and non-red seedlings would be expected in the ratio 3 : 1. Similarly, from back-crossing non-red to heterozygous red base, the two types of seedlings would be expected to occur in approximately equal numbers. From such crosses, however, in a total of 525 seedlings (10 crosses) no non-red seedlings appeared.

One direct cross between non-red plants of the two lines was made at the same time. This resulted in a small family of 5 seedlings, 4 of which were of the red base type and one non-red. This unexpected result led to further crosses of the same type, and 471 seedlings were obtained in 8 families. One of these seedlings was too weak to be classified, while all the others were red base. Thus only one non-red seedling was obtained from crosses which would be expected, were the colour factor in the lines identical, to produce only non-red progeny.

A few points remain to be investigated before the question of the origin of the odd non-red seedling (reference number 282/4) can be fully discussed, but it has already been ascertained that its occurrence has no special significance in connection with the general problem, and the results therefore lead to the hypothesis that we are here dealing with a normal case of complementary factors. On this hypothesis the non-red plants of Line 169 may be indicated by means of the zygotic formula **ccRR** and those of Line 174 by **CCrr**, and from inter-crossing these two types the double heterozygote **CcRr** should be produced.

Some plants from crosses of type **CCRr** × **ccRR** have been tested by selfing, and the two genotypes to be expected, **CcRR** and **CcRr** have been definitely identified.

Six plants obtained from the direct cross **ccRR** × **CCrr** when selfed gave red base and non-red seedlings in the proportions 174 and 150 respectively. This result shows a deviation of 8 seedlings (excess of non-red) from expectation on a di-hybrid basis. A seventh plant (382/3), however, when selfed gave a very considerable excess of non-red seedlings, and since it was far more highly self-fertile than the other plants, the results for this family cannot be included in the aggregate without unduly obscuring the fact that in the other families the agreement with expectation was satisfactory¹. It is obvious, however, that here again at least two independent factors are indicated, so that in this sense there is full agreement.

Similar abnormal ratios have not been obtained in further work. In 10 crosses of F_1 plants expected to be **CcRr** with either **ccRR** or **CCrr** non-red plants, the deviation from expectation on a 1:1 basis was 12.5 (excess of non-red) in a total of 681 plants. Red base plants from these crosses might be **CcRR**, **CCRr** or **CcRr**, and plants giving simple mono-hybrid and those giving the di-hybrid type of segregation have been identified by selfing (1616 classifiable seedlings).

Extracted non-red seedlings, sister plants to the foregoing gave, on selfing, non-red progeny only and by back-crossing to other plants of known constitution (mainly non-red of the two parental types), the three types **ccRR**, **CCrr** and **Ccrr** were definitely identified. The type **ccrr** would not be expected to occur amongst these plants, but the type **ccRr** which might be expected was not identified probably owing to the relatively small number of plants tested.

In the investigation of Plant 282/4 (referred to above) which has been proceeding side by side with that on the main problem, 32 crosses involving 1841 seedlings have already been made, and it has been proved that this plant is of the zygotic formula **Ccrr**. Further, in the course of this work the two non-red types **ccRr** and **ccrr** have been identified.

The hypothesis that complementary dominant factors, **C** and **R** are concerned with the production of red base-colour has, therefore, been fully confirmed. In the absence of either of these, no red base-colour

¹ This particular plant, 382/3, has been as yet little used in further breeding and the possible significance of the large deviation in this case has not been investigated.

is developed, and *Lolium perenne* is now added to the already long list of species for which similar colour-factor results have been obtained.

My acknowledgments are due to Mr A. R. Beddows, B.Sc., for patient assistance in the tedious work of hand emasculation and pollination.

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GENETIC STUDIES IN POULTRY.

VIII. ON A CASE OF SEX-LINKAGE WITHIN A BREED.

By R. C. PUNNETT, F.R.S. AND M. S. PEASE, M.A.

(With One Plate.)

IN an earlier communication¹ we referred to the existence of two genetically distinct types of transverse barring on the feathers of poultry, viz. that of the Plymouth Rock type which is dominant to self-colour and sex-linked, and that of the Hamburg-Campine type which is recessive to self-colour and not sex-linked. We stated further that we had started a series of experiments with the idea of discovering how these two types of rhythmical barring were related to one another, and it is with one of the results of these experiments that the present note is concerned.

We felt that the first step in our investigation was to make a variety which should be genetically pure for both kinds of barring: in other words, to add the dominant sex-linked barring factor to an otherwise pure strain of recessive barred birds. This we proceeded to do in a purely empirical way, starting by crossing Plymouth Rock with Gold Campine. F_1 birds with dominant barring were then mated back with pure Gold Campines, and of the resulting progeny the dominant barred individuals with most resemblance to the Gold Campine were again mated back with the pure Gold Campine. After several generations of this procedure we eventually obtained birds which, when mated with pure Campines, gave only birds of their own type and birds of the pure Gold Campine type. Genetically such birds only differ from pure Gold Campines in being also heterozygous for the dominant sex-linked barring factor (**B**) such as occurs in the Plymouth Rock.

The effect of this factor in the heterozygous state is to produce a peculiar form of barring to which we have applied the term "mixed." A few typical feathers showing "mixed" barring are illustrated on Pl. XVII, fig. 1, but beyond indicating their general appearance we do not at present propose to enter further into any discussion of the influence of the one type of barring or the other. In the cock, when hen-feathered, as is frequently the case, the feathers are of the same general type as in the hen. It will be noticed that the gold in the feather, where present, is

¹ *Journ. Gen.* 1921, XI, 235-40.

paler than in the pure Campine. This is especially noticeable in the gold neck hackles, and is doubtless due to some inhibitory influence exerted by the sex-linked barring factor on the formation of this pigment¹. In the down (cf. Pl. XVII, fig. 2) such mixed barred birds resemble the pure Campine, but, as would be expected, they show a light head patch in the occipital region, and at the same time the colour throughout is rather paler.

Having obtained mixed barred birds of both sexes, our next step was to breed them together. Since they were all heterozygous for **B**, about one quarter of their offspring were normal Campines, and this was already evident in the down. A larger proportion of the chicks came with the parental type of down (Pl. XVII, fig. 2), but about one quarter of the total showed a very much paler down as illustrated on Pl. XVII, fig. 3. From three pens of mixed barred birds (3 ♂♂ and 4 ♀♀) we bred 108 chicks in 1928, and these were distributed among the three classes of down as follows:

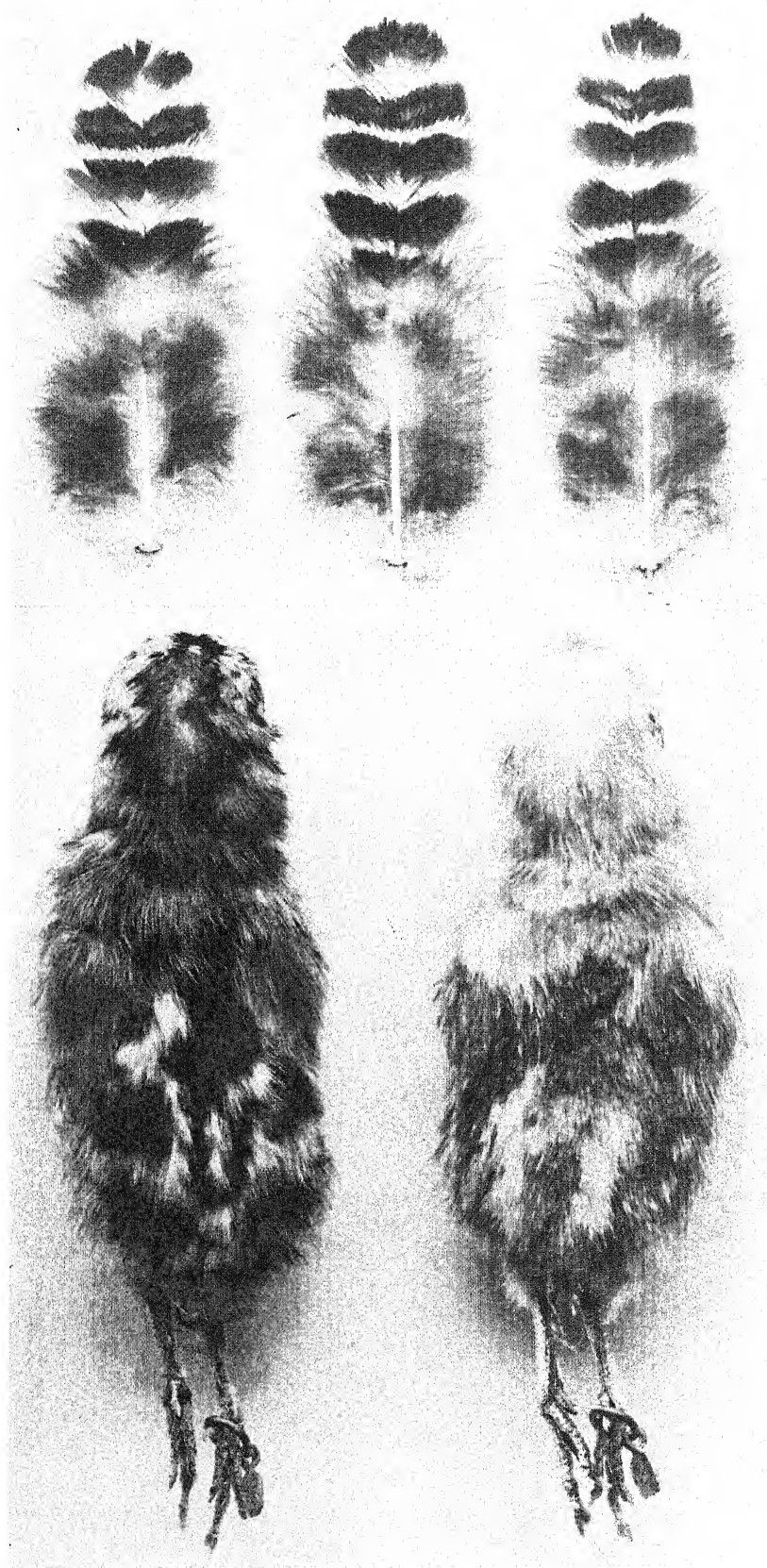
Normal Campine down	31
Campine down with light head patch			53
Pale "blotchy" down...	24

Of the chicks with pale "blotchy" down the sex was determined in 12 cases, and in every case was male. The sex of those with normal Campine down, where determined, was always female. Both sexes occurred among the chicks that came with a light head patch on Campine down.

Since the parents were all **Bb** in constitution, the simplest interpretation was to suppose that the chicks with pale blotchy downs were all derived from **B** eggs, which must carry "maleness," fertilised by a **B** sperm. In respect of the barring factor they should therefore be **BB** in constitution.

In the following year (1929) this point was tested by mating two of these presumably **BB** ♂♂ with mixed barred (**Bb**) ♀♀. Of the 33 chicks obtained 15 were of the pale blotchy type (Pl. XVII, fig. 3) and 18 of the Campine type with light head patch (Pl. XVII, fig. 2). Of the former type the sex was determined in 10 cases, and of the latter in nine cases. As was expected, the former were all ♂♂ and the latter all ♀♀. A cockerel which was pale blotchy in the down was also mated with a pure Gold Campine and, as expected, produced only chicks of the Campine type with the light head patch. And here it may be mentioned that the **BB** ♂

¹ Cf. R. C. Punnett and M. S. Pease, *Journ. Gen.* 1928, xix, 349.



is rather paler than the **Bb** bird, with a marked tendency to white wing feathers. It is, of course, well known that the **BB** Plymouth Rock male is of rather lighter type than the **Bb** bird—that even on a full black basis the inhibitory effect of **B** is greater in double than in single dose. The present case shows that on a basis which is not full black the disparity in the effect of a double and a single dose of **B** is much more marked, so that the sexes are different in appearance at all stages. It is, however, in the down that the disparity is greatest, and its effect has in this case led to the formation of a pure-breeding race in which the sexes are very clearly distinct at hatching. Economically this is likely to prove of considerable importance.

EXPLANATION OF PLATE XVII.

Fig. 1. Three feathers from the saddle of a mixed barred hen.

Fig. 2. Down of a mixed barred (**Bb**) pullet. Such a down is near that of a Gold Campine but rather paler and with a light head patch.

Fig. 3. Down of a mixed barred (**BB**) cockerel showing the pale "blotchy" type.

CHROMOSOMAL ABERRANTS AND GENE MUTATIONS IN *NICOTIANA* OBTAINED BY GRAFTING.

By DONTCHO KOSTOFF¹.

(With Twenty-eight Text-figures.)

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INTRODUCTION.

FOR the purpose of carrying on investigations on acquired immunity in plants (Kostoff, 1929) and on the histology of the callus (Kostoff, 1928) certain intergeneric graft unions were made. It was observed that irregular meiosis occurred in the pollen mother cells of the scions of certain combinations: viz. *Nicotiana Tabacum* grafted on *Datura Wrightii*, *Nicotiana Langsdorffii* grafted on *Solanum nigrum*, and *Petunia violacea* grafted on *Solanum nigrum*; and when the anthers of such scions were opened there was a significant percentage of abortive pollen grains. The flower buds of those scions with abortive pollen grains were selfed, and

¹ The author was a Fellow of the International Education Board from Sofia University when the main part of the work was done.

seeds were obtained that germinated and developed plants among which aberrants and mutants occurred. The production of these aberrant plants, their appearance, and their behaviour will be described here.

N. Tabacum was inbred by selfing for two generations, *N. Langsdorffii* for three, and *P. violacea* for one, without any noticeable variations appearing among their progeny.

METHODS.

(a) Grafting.

All of the grafting methods described by Winkler (1924) were used, but it was most frequently after whip grafting and tongue grafting that abnormal meiosis was observed in the scions. Numerous experiments convinced me that the meiotic irregularities were not dependent on the method of grafting, provided that a good join was formed between scion and stock.

(b) Cytology.

Some of the material for the cytological investigations was fixed in Bouin's solution as modified by Allen, and some was fixed in the following formula:

75 c.c.	Saturated solution of picric acid.
25 "	Formol.
10 "	Acetic acid.
1.5 gm.	Chromic acid.
1.0 "	Urea.

In the material fixed with the latter solution the chromosomes in meiotic and mitotic figures were much more distinct and easier for counting than those in material fixed in the former, but the prophase did not stain well. The permanent preparations were stained with iron alum haematoxylin. Temporary smear preparations were also made from the anthers of the plants studied.

APPEARANCE OF THE GRAFT UNIONS.

(a) *Nicotiana Tabacum* grafted on *Datura Wrightii*.

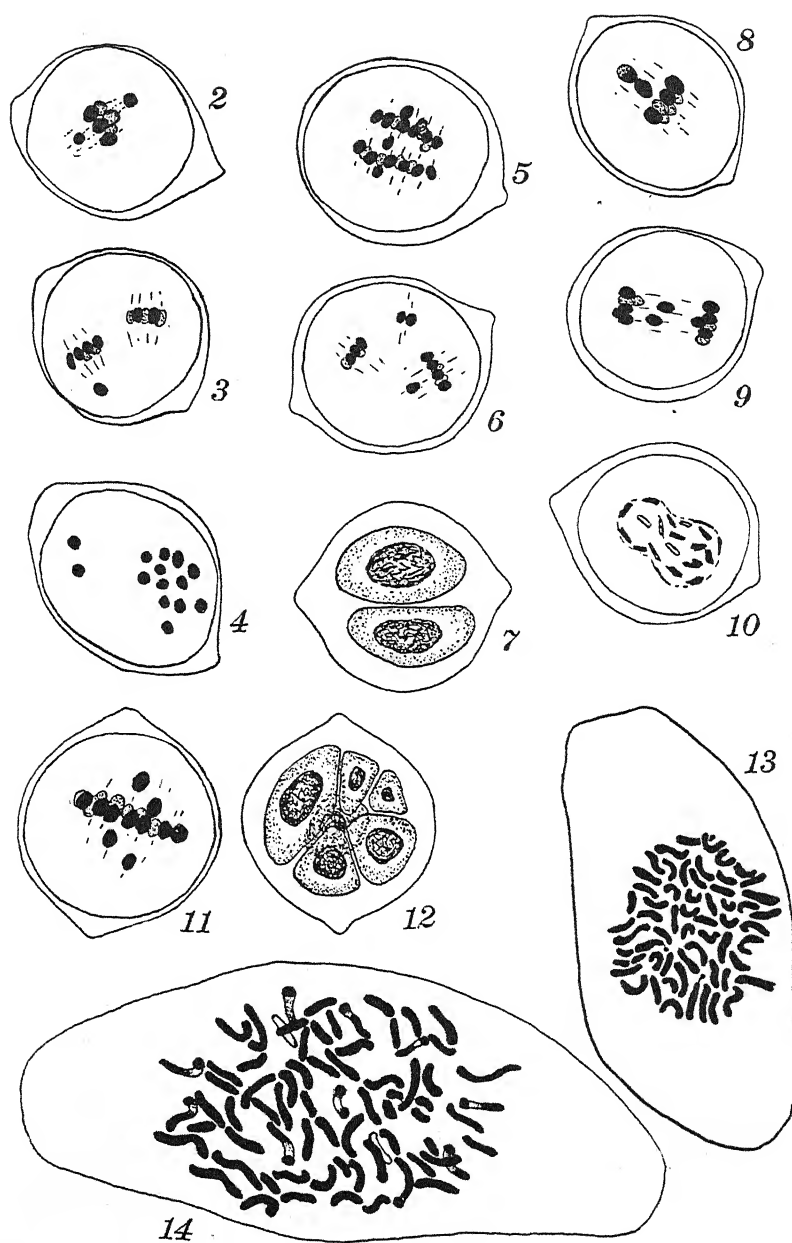
When *N. Tabacum* was grafted on *D. Wrightii*, the scions grew fairly normally, especially if an equilibrium was maintained between the mass of the scion and that of the stock. The flowers, however, were unable to develop normal corollas, and the apex of the sepals was usually more or

less destroyed (Fig. 1). In periods of intense sunshine, with a temperature of about 30° C., and an unsatisfactory water supply, all petals, as well as the greater part of the sepals, were shrunk and destroyed. With an adequate water supply and cloudy weather over a period of 10 to 12 days; the scions developed almost, but not quite, normal flowers.



Fig. 1. *N. Tabacum*: left, shoot grafted on *D. Wrightii*; right, non-grafted shoot.

The highest degree of disturbance in the corolla and calyx was accompanied by disorder in the meiotic divisions of the pollen mother cells (P.M.C.). The non-grafted *N. Tabacum* plants from which the grafted shoots were taken grew normally in the same environmental conditions with the grafted shoots, showed normal meiotic divisions in the P.M.C., and had normally developed pollen grains. On the other hand, the shoots grafted on *D. Wrightii* showed about 25-30 per cent. of abortive pollen grains, and corollas entirely destroyed. The appearance of the abortive pollen grains was the result of abnormal meiotic divisions.



Figs. 2, 3, 4. Disturbances in the reduction division in the P.M.C. of *Petunia violacea* grafted on *Solanum nigrum*.

Fig. 2. First meiotic division.

Fig. 3. Second meiotic division.

Fig. 4. Homeotypic metaphase with 2 chromosomes in one group and 12 in the other. The unequal distribution is due to non-disjunctions.

See foot of next page.

(b) *Nicotiana Langsdorffii* grafted on *Solanum nigrum*.

Successful graft unions of this combination were more difficult to secure than in the preceding combination. To obtain a successful growth of the scion it was necessary to cut off the shoots forming on the stock below the union, even though few leaves were left as a result of such pruning. When the scions formed flower buds very near the callus plane, as they often did, abortive pollen grains appeared in their anthers. One such flower bud, formed at a distance of only a few layers away from the callus, had about 70 per cent. abortive pollen grains in its anthers. The flower buds that developed at a great distance from the callus formed normal pollen grains.

(c) *Petunia violacea* grafted on *Solanum nigrum*.

P. violacea was easily grafted on *S. nigrum* by cleft grafting. Shoots of 3-5 cm. were usually chosen for such purposes. They grew on *S. nigrum* stocks 4 or 5 months, and in some cases reached a length of 30 or 35 cm. The *P. violacea* scions formed about 12-15 per cent. abortive pollen grains, while the plants from which the scions were cut formed only 1-0.5 per cent. The environmental conditions that increased the percentage of abortive pollen grains in the graft unions of *N. Tabacum* on *D. Wrightii* acted in the same way on *Petunia* scions where, occasionally, the percentage was increased to 35 or 40 per cent.

IRREGULAR MEIOSIS IN THE GRAFTED SHOOTS.

The meiotic divisions in the plants from which the shoots were taken for grafting were normal in all instances, in contrast to the disorder found in these divisions in certain of the grafted shoots.

The irregularities observed in the meiotic figures of the grafted shoots

Figs. 5, 6, 7, 8, 9, 10. Irregular meiosis in the P.M.C. of a *N. Langsdorffii* grafted on *S. nigrum*.

Fig. 5. Homeotypic anaphase with double number of chromosomes and one spindle following an interkinesis of the type shown in Fig. 10.

Fig. 6. Homeotypic division with three spindles appearing when the chromosomes of both poles and those lagging on the spindle undergo interkinesis separately.

Fig. 7. Dyads with double number of chromosomes (18) formed after a division such as illustrated in Fig. 5.

Fig. 8. Early metaphase with a single chromosome leading the advance to the poles.

Fig. 9. Late anaphase with chromosomes lagging on the spindle.

Fig. 10. Interkinesis including chromosomes of both poles and those lagging on the spindle.

Fig. 11. Irregular heterotypic division in P.M.C. of *N. Tabacum* grafted on *D. Wrightii*.

Fig. 12. Hexad formed in *N. Tabacum* when this plant is grafted on *D. Wrightii*.

Figs. 13, 14. *N. Tabacum* aberrants obtained after selfing the scion grafted on *D. Wrightii*.

Fig. 13. Metaphase from a root tip of an aberrant plant with 59 chromosomes.

Fig. 14. The same of an aberrant plant with 72 chromosomes.

were similar in all cases of the three combinations studied, so that the details described are applicable for the scions of the three unions. Some meiotic phases are illustrated in Figs. 2-12 inclusive. They remind one of the meiotic divisions in species hybrids (Kostoff, 1930) and of those in certain flowers attacked by parasites (Kostoff and Kendall, 1929 *a, c*). In early anaphases (Figs. 2, 8, 11) single chromosomes usually lead the advance to the poles, and in the late anaphases (Fig. 9) a number of chromosomes very often lag on the spindle. Non-disjunctions were often observed during both divisions. As a result of such irregularities, plates, like those in Fig. 4, were found during the homeotypic metaphase with various numbers of chromosomes. The figure just referred to illustrates the second meiotic division in a P.M.C. of a *P. violacea* scion with 2 chromosomes in one plate and 12 in the other.

In some P.M.C.'s the chromosomes are spread out over the entire spindle, and undergo interkinesis together (Fig. 10). After such an interkinesis the chromosomes sometimes fail to become organised into a normal equatorial plate preparatory to the second division, but divide and form monads. When a comparatively normal plate, containing all of the chromosomes ($2n$ chromosomes), forms preparatory to the second division, dyads result (Figs. 5, 7). When one or more of the chromosomes go through the interkinesis apart from the two polar groups, a third spindle is formed for it or them during the second division (Fig. 6), and sextads result (Fig. 12). Triads, pentads, and octads have also been observed as products of irregularity of chromosome distribution. About 30 per cent. of the pollen grains were abortive in *N. Tabacum* scions, about 50 per cent. in the *N. Langsdorffii* scions, and about 35-40 per cent. in the *P. violacea* scions. The pollen grains that stained red in acetocarmine were considered viable, since grains of this type germinated in stigmal secretion. These viable pollen grains varied greatly in size and apparently contained from n to $4n$ chromosomes. Some of the largest ones, perhaps representing monads ($4n$ chromosomes), often germinated in stigmal secretion with two and occasionally with three pollen tubes.

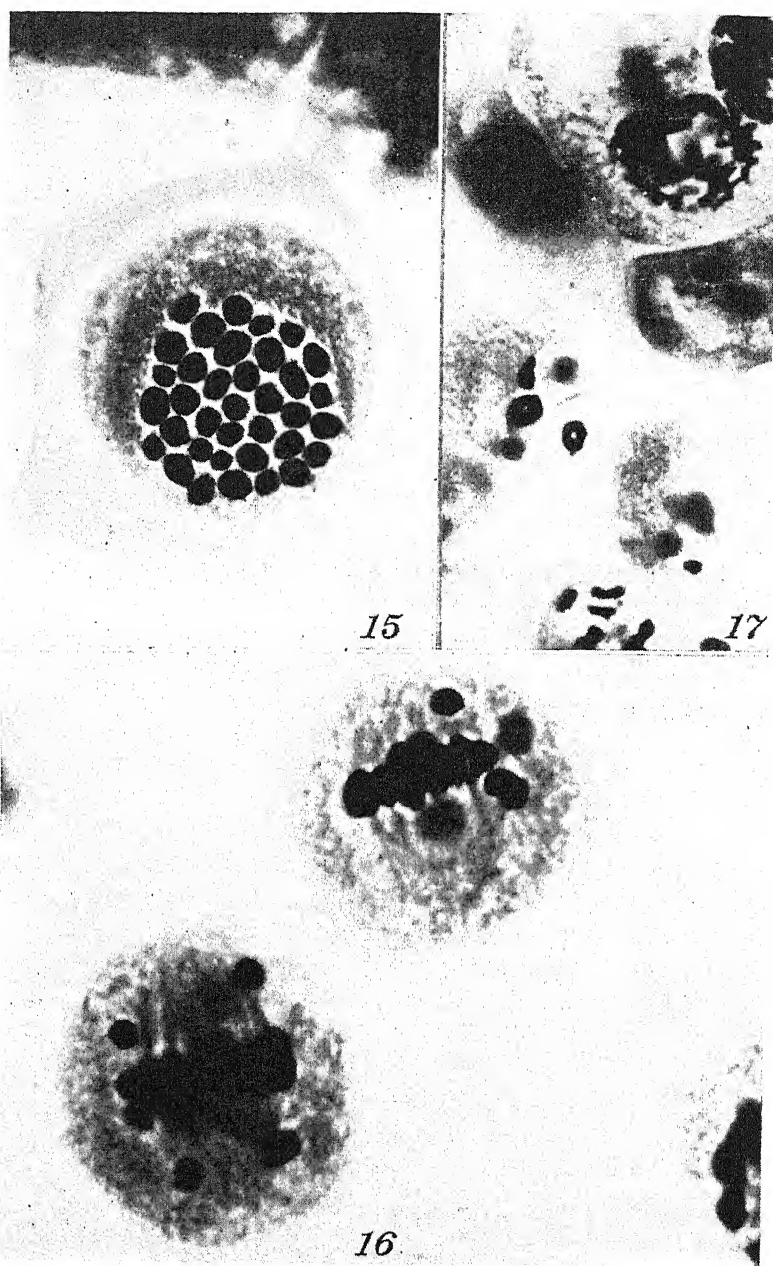
From the selfed buds of scions of *N. Tabacum* and *N. Langsdorffii* seeds were obtained that germinated well and developed mature plants. These plants are described under the following headings.

F_1 GENERATION.(a) *Morphology and cytology of the F_1 generation of N. Tabacum scion.*

Seventy-eight plants were raised from the seeds obtained after selfing the buds of *N. Tabacum* scions with 30 per cent. abortive pollen grains. Of this number 76 appeared entirely normal, while 2 differed slightly morphologically and had a high percentage of abortive pollen grains. I shall refer to these two plants as plant "G" and plant "D."

Plant "G" was somewhat larger in size than the other 76 plants, had slightly broader leaves, a smaller and darker corolla, and produced very many flowers. It flowered 8 to 9 days earlier than the other plants. In the root tips of this plant 72 chromosomes were counted most frequently (Fig. 14), but in some plates 70 and 71 chromosomes were observed. It was difficult to determine the exact number with so many chromosomes present, but I believe that the correct number is 72. In the cases where 70 and 71 were observed, one or two of the chromosomes may have been removed by the knife, or they have been so close together that two were counted for one. In the P.M.C. of plant "G" 36 chromosomes were usually observed (Fig. 16), but the gemini were very loose, so that some of the chromosomes separated very early (Fig. 16), and 37-39 chromosomes were often seen. The chromosomes varied greatly in size in the heterotypic metaphase, as one can see in Fig. 15. Whether all of these chromosomes were bivalent, or whether some of them were univalent and others trivalent, was a difficult question to decide. Judging from the size, one might suppose that univalent and trivalent chromosomes were also formed in addition to the bivalent ones. This seems a plausible assumption, for trivalency has been observed in *Nicotiana* (Kostoff, 1930). The meiotic divisions were irregular, and the abnormalities that have already been described in the scions were also found in plant "G." I should classify the type of irregularities in the meiotic figures of this plant, and in the scions, in Group 3, with several chromosomes lagging on the spindle during the heterotypic division in the scheme offered in a previous publication (Kostoff, 1930). Plant "G" was partially fertile after selfing it, and in pollinating it with the normal *Tabacum* plants.

Plant "G" apparently originated from the fusion of an egg cell having 24 chromosomes ($n = 24$ in *N. Tabacum*) with a sperm nucleus from a dyad pollen grain that had 48 chromosomes ($2n$) or, perhaps, from a fusion of an egg that had 48 chromosomes as the result of irregular



Figs. 15, 16. Microphotographs of P.M.C. from *N. Tabacum* aberrant with 72 somatic chromosomes.
 Fig. 15. Metaphase (36 chromosomes).
 Fig. 16. Early anaphase.
 Fig. 17. Parasynopsis in *N. Langsdorffii*.

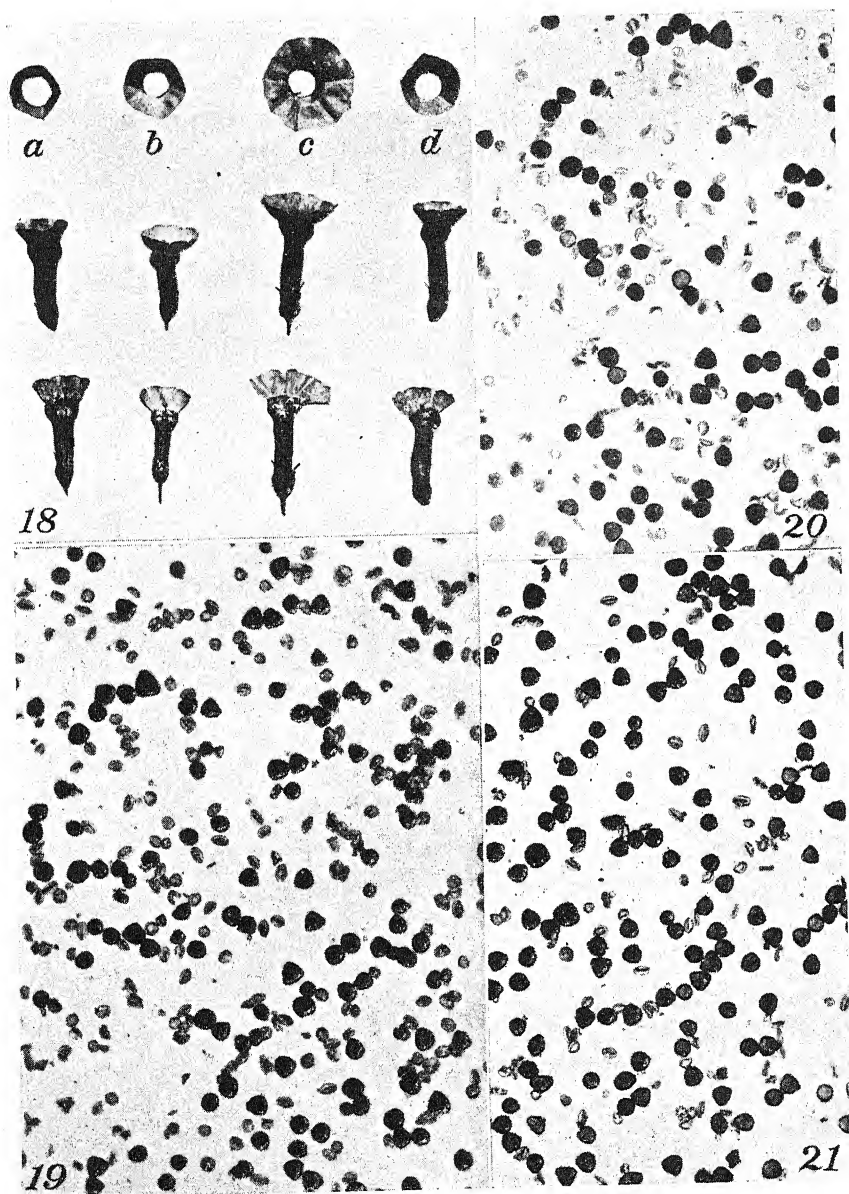


Fig. 18. Flowers from *N. Langsdorffii*: (a) plant 1000 that had 18 somatic chromosomes and about 50 per cent. abortive pollen grains (see Fig. 21); (b) plant 962 that had 52 somatic chromosomes and about 75 per cent. abortive pollen grains (see Fig. 20); (c) plant 1004 that had 21 somatic chromosomes and about 65 per cent. abortive pollen grains (see Fig. 19); (d) normal plant.

Fig. 19. Pollen grains from plant 1004, about 65 per cent. abortive.

Fig. 20. Pollen grains from plant 962, about 75 per cent. abortive.

Fig. 21. Pollen grains from plant 1000, about 50 per cent. abortive.

meiosis in the embryosac mother cells of the scion with a normal sperm nucleus having 24 chromosomes.

Plant "D" flowered about 8 to 10 days after the other 76 sister plants. It was smaller than the other, had a larger corolla with a lighter colour than in the other plants, leaves that were smaller and more elongated, and tended to teratological proliferations. In the root tips of this plant 59 chromosomes were counted (Fig. 13). In the heterotypic metaphase of the P.M.C. 35 to 40 chromosomes of various sizes were observed, the variation resulting perhaps from the loose connection of the gemini. The meiotic divisions were more irregular than those in plant "G," but of the same type. Plant "D" was slightly self fertile.

Eighty plants were raised from seeds of the normal *N. Tabacum* plant from which the grafted shoots were taken. These plants were uniform, and no abnormal ones were found among them.

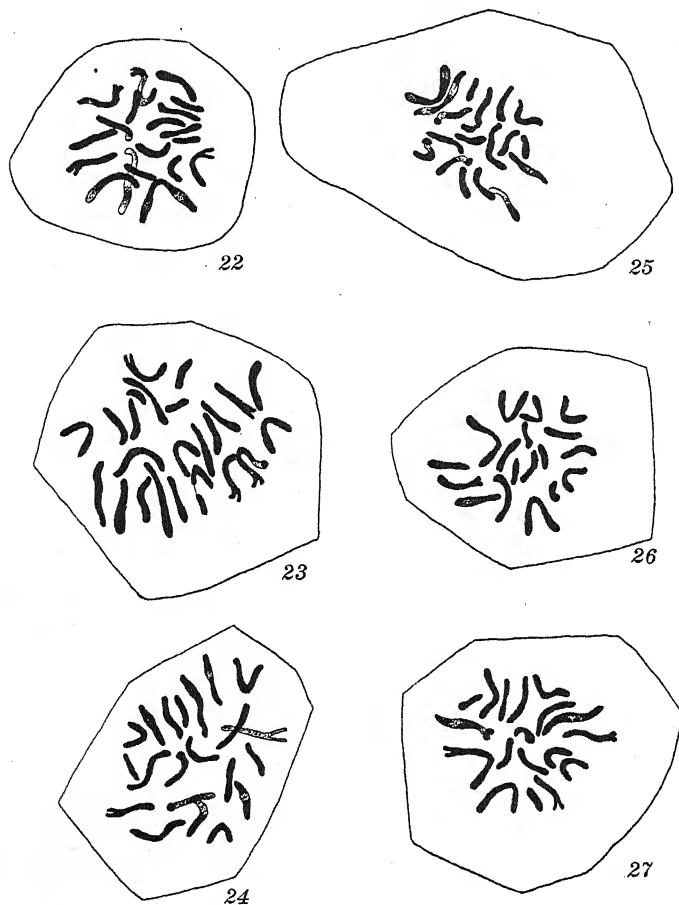
(b) *Morphology and cytology of the F_1 generation of N. Langsdorffii scion.*

From seeds obtained after selfing the buds of the *N. Langsdorffii* scion with 50 per cent. abortive pollen grains, 420 plants were raised. Among this progeny there were 12 plants that had a high percentage of abortive pollen grains. Of these 12 plants, 9 were dwarf, grew very slowly, and began to flower about 20 to 25 days after the other 411 plants. Three of these *Langsdorffii* dwarf plants (nos. 913, 962 and 1000) and the other three plants, which were normal in size but had a high percentage of abortive pollen grains (nos. 1002, 1003 and 1004), were studied more thoroughly. Some of them differed morphologically from the normal ones in the size, shape, and colour of their foliage leaves and petals. Plants 1000, 962 and 1004 (Figs. 18 (a), (b), (c)) showed the most striking deviations from the normal plants (Fig. 18 (d)). Plant 1000 had short, succulent leaves and slightly shorter and broader flowers than the normal (Fig. 18 (a)). Plant 962 had small yellowish leaves and small pale flowers (Fig. 18 (b)), while plant 1004 had large, broad leaves and large flowers with a very broad corolla (Fig. 18 (c)).

Plants 913, 1000 and 1002 had about 50 per cent. abortive pollen grains (Fig. 21); plants 1004 and 1003 had about 65 per cent. abortive pollen grains (Fig. 19); and plant 962 had about 75 per cent. abortive pollen grains (Fig. 20).

Plants 1000, 1002 and 913 had 18 somatic chromosomes, *i.e.* the normal chromosome number of the mother plant. Plant 1003 had 19 somatic chromosomes, *i.e.* it was a trisomic plant (Fig. 22); plant 962

had 25 somatic chromosomes (Fig. 23); and plant 1004 had 21 somatic chromosomes (Fig. 24).



Figs. 22-27. Somatic metaphases in the root tips from *N. Langsdorffii* plants.

- Fig. 22. Plant 1003 with 19 somatic chromosomes.
- Fig. 23. Plant 962 with 25 somatic chromosomes.
- Fig. 24. Plant 1004 with 21 somatic chromosomes.
- Fig. 25. Plant 1000 with 18 somatic chromosomes.
- Fig. 26. Plant 1002 with 18 somatic chromosomes.
- Fig. 27. Plant 913 with 18 somatic chromosomes.

Both meiotic divisions were irregular in the six plants studied. In the P.M.C. of plant 913 no pairing of the chromosomes occurred, while in the mother plant and in the other plants studied very distinct figures of parasynthetic pairing were observed (Fig. 17). This plant was sterile.

Plant 962 showed very low fertility, plants 1004 and 1000 were partially fertile, and plants 1002 and 1003 were fully fertile.

For control 200 plants were raised from seeds obtained from those plants from which the scions were taken. No noticeable variation was observed among these plants, and none of them had abortive pollen grains like those plants found among the progeny of the scions.

F_2 GENERATION.

(a) *Morphology and cytology of the progeny of Tabacum plant "G."*

Ninety-two plants were raised from the seeds obtained after selfing plant "G." These plants showed a great diversity in size, shape, and colour of the organs. Some were dwarf, some were very tall, and others ranged between these two extremes. The foliage leaves varied in colour from a dark to a very light green, from those extremely succulent to those very thin. In general outline these leaves showed many variations: linear, lanceolate, oblong, ovate, elliptic, spatulate, and obovate; and in many the borders were curled owing to uneven growth. Some leaves were sessile, other petiolate; in the latter case the petioles also showed a diversity of size and form. In some instances leaves like those of normal *N. Tabacum* were displayed. The flowers likewise exhibited variations in size, shape, and colour from flowers like those of the normal *N. Tabacum* plant; some had flowers twice as large as those with the smallest, and the colours varied from light rose to dark red. The petals differed in size, so that the angle in the apex varied from 30-32° to 120-125°.

The chromosome number and behaviour during the meiotic divisions in the P.M.C. was studied in acetocarmine for 18 plants of this progeny. The results of the chromosome counts are given in Table I. Some of the plants were fully fertile, others only partially so.

TABLE I.

No. of plants studied	Chromosomes counted	
	In heterotypic metaphase	On both poles, late anaphase
8	24	48
2	25-26	50-51
1	27	52-53
3	32	About 60
2	34-35	" 62
1	36-38	" 70
1	40-42	" 78

The data given in Table I show that the triploid *Tabacum* plant produced progeny with very variable numbers of chromosomes, a condition apparently the main cause for their morphological variation. The latter depends not only on the number of the chromosomes but also on which ones are present. In addition to this there are, perhaps, the gene mutations, that can occasionally occur. Among the progeny there was even one hypertriploid.

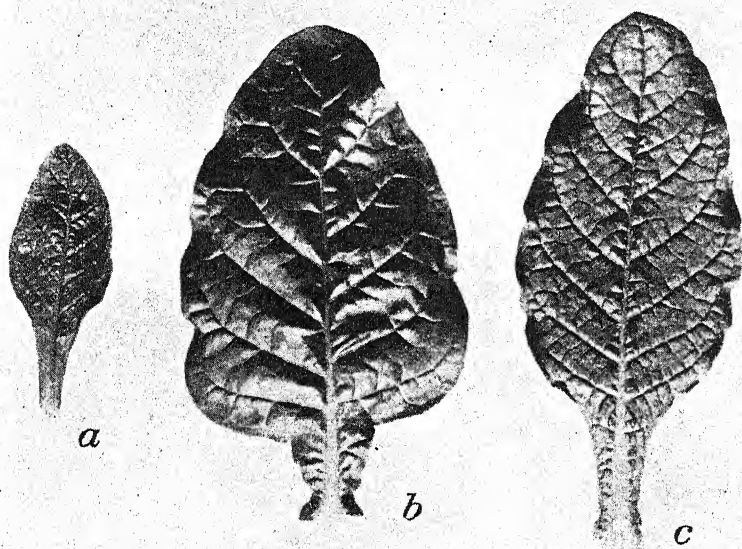


Fig. 28. Leaves from *N. Langsdorffii* plants: (a) plant 1003/22; (b) plant 1003/30; and (c) a normal plant.

(b) *Morphology and cytology of the progeny of Langsdorffii plants 1002 and 1003.*

Plant 1003 gave progeny that were extremely variable. Out of 50 plants, one (no. 1003/22) had very small leaves. When the leaves of this plant were young they grew normally, but when they reached the size shown in Fig. 28 (a) (compare with the normal leaf given in Fig. 28 (c)) the growth ceased, and they remained about one-half the size of the normal ones. Although old, these leaves look like young leaves, for the expansion of the leaf cells has not taken place. The leaf given in Fig. 28 (a) is about 15 days older than the normal *Langsdorffii* leaf given in

Fig. 28 (c). There were 17 chromosomes on both poles in the P.M.C. during the late anaphase; obviously, the plant was monosomic.

Another plant, 1003/30, presented an interesting case where the deviation from the appearance of the mother plant was limited to the type of leaf, which was very broad and juicy (Fig. 28 (b)). The flowers were normal, entirely normal pollen grains were formed, and the normal number of chromosomes was present ($n = 9$, $2n = 18$). The character broad, succulent leaves was apparently an hereditary one, for the progeny of this plant all (25 plants) had broad, succulent leaves. For this reason I am inclined to call plant 1003/30 a mutant.

The progeny of plant 1002 presented a feature of even greater interest. I raised 62 plants from seeds obtained from selfing plant 1002. These all had the appearance of normal *Langsdorffii* plants except in the character of pollen colour. Plant 1002 had blue pollen grains, as all *N. Langsdorffii* plants do, only they were not as deeply coloured as those of normal plants. Among its progeny there were 16 with white pollen grains, 29 with pollen grains like the mother plant (1002), and 17 with very deep blue pollen grains as in entirely normal *Langsdorffii* plants. Blue colour in *N. Langsdorffii* is a dominant character, but perhaps not completely dominant, for F_1 (*N. Langsdorffii* \times *N. Sanderac*¹) plants in heterozygotic condition have blue pollen grains like plant 1002, where the colour is not as dark as in the homozygous plants. In these progeny of plant 1002 the Mendelian ratio of the plants with white pollen grains corresponds closely to the behaviour of a simple Mendelian recessive character. The appearance of *N. Langsdorffii* plants with white pollen grains was obviously due to gene mutation that apparently occurred during the meiotic divisions of *N. Langsdorffii* scions, and was carried by plant 1002 in heterozygotic condition as a recessive character.

DISCUSSION.

The observations reported herein present a series of problems and questions that need answers, but about which very little can be said at the present time when physiogeny is just beginning to develop.

The first two questions to be discussed are: (1) what causes the meiotic irregularities in the P.M.C. of the scions? and (2) how do these irregularities occur?

Many investigators have already reported that various agents can cause irregular meiotic or mitotic cell division. I shall recall only a few typical instances. Němec (1903) produced irregular mitoses in plants by

¹ *N. Sanderac* has white pollen grains.

narcotics. Alberti and Politzer (1923) observed it in corneal epithelium of *Salamandra maculosa* after treatment by X-rays. Abnormal mitosis was also observed by Němec (1924) and Kendall (1929) in mite galls, by Kostoff and Kendall (1929 *a*) in Cynipid galls on *Quercus*, and in the Cynipid pupae where it is apparently caused by the autolytic processes, by the author in hybrids and hybrid tumours (1930), and by Winge (1927) in beet cancer. Abnormal reduction division and formation of generative cells and pollen grains with increased numbers of chromosomes has been observed by Sakamura and Stow (1926) in *Gagea lutea* after extreme changes in the temperature, by Kostoff and Kendall in *Lycium* (1929 *a*) and *Datura* (1929 *c*) as the result of injury from mites, by Goodspeed (1929) in *Nicotiana* after exposure to X-rays, by Packard (1916) in *Nereis* egg cells, and by Mohr (1919) in *Decticus* during the spermatogenesis after exposure to X-rays. Irregular meiosis in species hybrids is a very common and well-known phenomenon.

An attempt to generalise from these observations brings out the fact that the causes of abnormal mitotic and meiotic divisions are various chemical and physical agents¹ or factors that very seriously affect the cell dynamics. At first sight such a generalisation may appear too wide, but every agent that is able to divert some biochemical or biophysical process from its normal trend during the cell division must be considered as a possible factor in bringing about irregular cell division. In our case one must assume that the agents coming from the stock are the cause, or causes, for the irregular meiosis in the scion.

At first this postulate seems contradictory to the results of the experiments reported in a previous publication (1929 *a*) where it was stated that the scion could produce antibodies (chiefly precipitins) against the stock. From this one might suppose that specific substances of the stock would be precipitated in the callus region, and be unable to reach the flower buds, especially in the graft unions between *N. Tabacum* and *D. Wrightii* where the flower buds were formed a considerable distance from the callus region. But the precipitates and their disintegration products are foreign substances for the scion, and the despecification of the stock substances takes places progressively from the callus region to the top of the scion. The velocity of this process depends greatly on the velocity of the sap ascent, and on the amount of the destructive agents

¹ A sharp line cannot be drawn between the chemical and the physical, for chemicals cause deviations from the normal physical conditions in living cells even as physical agents cause directly, or indirectly, deviations from the normal chemical processes and thus change the end results.

present in the scion. In *N. Tabacum* scion the most pronounced irregularities in the meiotic figures occurred when the graft grew in intensive light and relatively high temperature (about 25–28° C.), so that intensive transpiration took place and the velocity of the sap ascent was increased. The sap brings with it, of course, in such conditions a greater amount of precipitates and their disintegrating substances that are still foreign for the scion cells than when the weather is cloudy and the temperature relatively low. When a rapid ascent of the sap occurs, it is possible that even some of the stock specific substances can reach the flower buds before they are attacked by the scion antibodies. This is always true for the non-antigenic substances of the stock that may reach the flower buds unchanged and act there as foreign substances so that irregularities are caused in the meiotic process.

At this point it seems relevant to consider another important condition. All solanaceous plants have a high content of alkaloids that differ in the various genera. After grafting, the alkaloids from the stock may penetrate into the scion, more or less according to the environmental conditions, and act like narcotics in the scion. And Bünning (1929) reported that “Narkotika zerstören die Semipermeabilität der Plasmahaut; das führt zur Permeabilitätserrhöhung. . .,” even if this increase is not a permanent one. Permeability also depends partially on the environmental conditions. I shall mention here the investigations of Brauner (1924) who reported, “. . .dass das Licht die Permeabilität der Avena-Koleoptile erhöht. . .,” and those carried out by Gellhorn and Gellhorn (1928) who reported that, “Der Temperaturquotient der Permeabilität ist also nicht einheitlich, sondern von der Natur der permeierenden Substanz und der Beschaffenheit der Zelle abhängig.” Magstris and Schäfer (1929) also demonstrated that the permeability is increased by high temperature (not above 30° C.), intense light, and foreign substances.

The second question for discussion, how the irregularities occur, has been thoroughly discussed elsewhere (Kostoff, 1930), and here we need only state the conclusion, viz.: the agents that attack dividing cells, or cells preparatory to division, cause physical, chemical, or physiochemical changes in the protoplasm resulting in certain disturbances in the division processes that are expressed chiefly by the retardation of all the latter, and by the irregular distribution of the chromosomes during cell division. In connection with the discussion of the activities of alkaloids and narcotics and those of the dynamics of the cell division I shall recall the opinion of Traube (1928), who writes: “Die Wirkungs-

mechanismus der Narkotika beruht auf der Hemmung chemischer, physikalischer und insbesondere bioelektrischer Vorgänge...." The observations made on the meiotic divisions in the p.m.c. of the scions, namely, that the whole division was retarded and led to formation of monads and dyads, confirm the opinion of Traube.

The chromosome separation process appears to be independent of the chromatoproteolytic process that occurs in late telophase and during interkinesis, for in some p.m.c.'s the chromatoproteolytic process often started during the heterotypic division, before the complete separation of the chromosomes had taken place. After such an interkinesis, only one spindle was formed during the second division, and dyads originated. When a great retardation of the second division also occurred, and the process of chromosome separation was not completed when the process of pollen formation began, monads were formed. The origin of the triploid *N. Tabacum* plant "G" apparently came about through the fusion of a normal egg with a sperm nucleus arising from a dyad pollen grain and so having 48 chromosomes, or *vice versa*, a diploid egg cell fertilised by a normal sperm nucleus ($n = 24$). The other abnormal plants have undoubtedly arisen from a fusion of two generative nuclei with abnormal chromosome number, or from a fusion of a normal generative nucleus with an abnormal one. Aberrant plants have been obtained by Gager and Blakeslee (1927) after exposures of young buds of *Datura* to radium preparations, and by Goodspeed (1929) in *Nicotiana* by the use of X-rays.

The mutant characters have also appeared, presumably, during the meiotic divisions in the scions, and have been carried further by the gametes of the scion. The character of white pollen has obviously been carried by plant 1002 in heterozygotic condition as a recessive character, to appear in the next generation as a pure white character in the ratio already mentioned. After the publication by MacDougal, Vail and Shull (1907) other instances of artificially induced mutations are those obtained by Pirovano (1922) in exposing pollen grains of various plants to electric currents, radium rays, ultraviolet rays, etc. Although his experiments were not arranged with such exact genetical accuracy or so cleverly as those of Muller (1928) on *Drosophila*, some of them are sufficiently convincing and deserve consideration. Mutations were also produced artificially in *Zea Mays* by the author (Kostoff, 1925). Two years later, Gager and Blakeslee (1927) reported their experiments on the production of mutations in *Datura* by radium rays. Recently, Goodspeed (1929) has given his attention to the problem, and in his laboratory mutations have been obtained by X-rays and radium rays.

From the instances cited it is obvious that mutations have arisen in plants under extremely abnormal conditions causing, in addition to the temporary physicochemical changes in the living cells, some permanent changes in the hereditary units. In the light of newer concepts the latter are possibly autocatalysators (Goldschmidt, 1927) of organic nature with relatively large molecules which, like large organic molecules in general, have many thousands, even millions of stereoisomeres. Under extreme conditions it is possible for such molecules to be changed. Such conditions offer all the agents by which mutations have been induced. Tischler's expression (1928): "Ändert sich mutativ ein Gen, so ist damit die Chromosomensubstanz qualitativ etwas umgestaltet. Und bei der nächsten Kernteilung schon kann gegenüber der bisherigen Form eine neue dasein, die ein wenig anders ist als bisher" is in agreement with the above ideas.

An explanation of the behaviour of the *N. Tabacum* triploid plant "G" can be given within the limits of Goldschmidt's physiological theory of heredity (1927). Plant "G" was not very noticeably larger than the others, but it matured 8 to 9 days earlier than the normal. It had one set of chromosomes more than the others, *i.e.* $3n$ instead of $2n$. Since the hereditary units are located in the chromosomes (Morgan, 1928) and since the "Schichtung" depends on the quantity of the substrates and catalysators, the velocity of the reactions has been highly increased, *i.e.* the determinative, differentiative, and formative processes have been brought about more rapidly.

The appearance of the dwarfs can be interpreted on the same basis. These were either aberrants or mutants, and in their organisms the ratio of the components for the "Schichtungen" has been unbalanced by the quantitative increase or decrease of only certain catalysators (located in the chromosomes present in excess, or in those missing) and substrates, thus bringing about a decrease in the velocity of the reactions.

There is, perhaps, one other point that deserves attention here. In the P.M.C. of *N. Langsdorffii* plant 913 no pairing of chromosomes was observed. Non-pairing of chromosomes due to a simple Mendelian gene was reported recently by Beadle and McClintock (1928). Apparently, the non-pairing in plant 913 was also due to such a gene or genes.

Recently, Daniel (1927 *a, b*) published his observations on the progenies from the selfed scions of graft unions in various Compositae. He found great variation among the progeny, especially among those of *Helianthus tuberosus* grafted on other species of the genus. He ascribes this variability to "specific influence," in the Lamarckian sense, of the

stock on the scion. He does not mention, however, any determination of the homogeneity of his *H. tuberosus*. If the scions were homozygous, the variants he observed may have been aberrants, mutants, or both, like those described here.

SUMMARY.

1. Irregular meioses were observed in the P.M.C.'s of the scions in certain intergeneric graft unions.

2. Such scions produced a high percentage of abortive pollen grains and pollen grains with increased numbers of chromosomes.

3. Chromosomal aberrants (monosomic, trisomic, triploid, hypertriploid, etc.) and gene mutations were found in the generations following the selfing of the flowers of the scions having abortive pollen grains. The controls gave only normal plants.

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THE MODE OF ORIGIN AND CHROMOSOME BEHAVIOUR IN POLLEN MOTHER CELLS OF A TETRAPLOID SEEDLING TOMATO¹

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(With Two Plates and One Text-figure.)

THE F_1 progeny from a cross between a double-trisomic (26-chromosomes) and diploid of the tomato variety Dwarf Aristocrat consisted of diploids, simple trisomics, a trisomic with an additional chromosome fragment, and one plant, number 27,048,10, which proved to be a tetraploid. This plant showed no evidence of injury such as might cause a diploid plant to become tetraploid.

Since the tetraploid seedling originated from parents belonging to similar lines of the same variety, its distinctive characteristics could hardly be due to recombination of genes. Winkler's⁽⁹⁾ account of the gross morphology of his "gigas" (tetraploid) yellow-fruited King Humbert applies almost entirely to our seedling. As Winkler remarks, the tetraploid is more than a mere "Mastexemplar" of the diploid (Plate XVIII, *a, b*; Plate XIX, *c, d*). The leaves of the tetraploid are darker green and larger, the leaflets broader, the petioles and stems thicker and the flowers larger than those of the diploid. In these respects the tetraploid resembles our triploids, but the differences between tetraploid and diploid are greater than between triploids and diploid. On the other hand, the tetraploid is more fruitful than the triploids, and in this respect is less distinct from diploids. Of the six fruits borne on the original tetraploid, two were deeply lobed; such lobing is uncommon on the diploid. When selfed, the tetraploid yielded viable seeds but not as many per fruit as the diploid. A small progeny of tetraploid selfed consisted of twelve plants similar to the tetraploid and one apparently distinct. No chromosome counts were made. Tetraploid \times diploid set

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fruit fairly readily. Nearly all the seeds in these fruits were abortive, but a few were of normal size and two of them have germinated. No viable seed was obtained from the reciprocal cross. Our tetraploid apparently grows more slowly than the diploid, although the gene balance in these two forms must be nearly identical. Contrary to Winkler's finding, the plastids of the tetraploid were not appreciably larger than those of the diploid. If tetraploid plants had previously occurred in our cultures, it seems certain that they would have been detected, since they are morphologically even more distinct from diploids than are triploids.

MODE OF ORIGIN OF POLYPLOID TOMATOES.

In our cultures at Riverside and at Berkeley, California, in the past six years, three triploids were found among about 9000 plants probably originating from diploid parents, but six triploids occurred among only about 2000 plants from trisomic parents. The occurrence of triploids (6) strongly suggests that diploid gametes can function. Apparently triploids are more likely to occur in the progeny of parents with unbalanced chromosome numbers than in the progeny of diploids. There is genetic evidence in one instance that a triploid has arisen from a diploid gamete which originated as a result of reduction in a tetraploid mother cell. Flesh colour of fruit depends on the allelomorphs **R** (red flesh) and **r** (yellow flesh). A simple trisomic (triplo-*C*) F_1 plant originating from triploid (**RRR**) \times diploid (**rr**) gave in F_2 a triploid with yellow flesh. Inheritance of flesh colour was disomic. If, as is probable from its recessive yellow colour, the triploid is **rrr** and not **Rrr**, it must have originated from **rr** and **r** gametes. The diploid gamete evidently originated in the reduction division of a tetraploid (**RRrr**) mother cell. In simple- and double-trisomic tomatoes we have occasionally observed tetraploid pollen mother cells, which have twice the volume of diploid pollen mother cells, and also groups of normal pollen mother cells which were undergoing non-reduction. The suggestion that triploids originate from the union of diploid and haploid gametes is apparently weakened by the difficulty met with in crossing tetraploid and diploid. It should be noted, however, that the tetraploid, even when selfed, sets seed less readily than the diploid. Tetraploid pollen mother cells in a diploid plant were always found to be in a less advanced stage of maturation than adjacent diploid pollen mother cells. This was not unexpected since the tetraploid islands in root tips apparently increased in size in harmony with the

surrounding diploid tissue, consequently the rate of division in the tetraploid is in this case half that in the diploid¹.

It is possible that, as a rule, diploid eggs are immature when reached by rapidly growing haploid pollen tubes, and that the diploid pollen tubes grow too slowly to reach haploid eggs before they are too old. This would explain the difficulty experienced in crossing tetraploid and diploid plants.

If the tetraploid seedling originated from the union of two diploid gametes, clearly the gametes did not originate by non-reduction, for in that case the two extra chromosomes of the double-trisomic ♀ parent would be present. Diploid gametes seem unlikely to have originated from doubling of the chromosome number prior to reduction, for, in all probability, there would have been $4N + 4$ chromosomes in each mother cell. As the four extra chromosomes would tend to form pairs or to unite with groups of four to form hexavalents, the loss of all the extra chromosomes during maturation seems improbable. On the other hand, the excess of diploid plants found in the progenies of simple- and double-trisomic tomatoes (5) may indicate that elimination of extra chromosomes during maturation of $2n + 1$ and $2n + 2$ mother cells is common. The mode of origin of the tetraploid seedling is uncertain, since it originated in a pure line, but probably it resulted from doubling of the chromosomes in a somatic cell very early in embryogeny. The tetraploid interspecific hybrid of *Nicotiana* originated in this manner according to Clausen and Goodspeed (3), also the tetraploid *Datura* plants (1).

CHROMOSOME BEHAVIOUR IN THE POLLEN MOTHER CELLS.

Winkler (9) and Jørgensen (4) have reported the occurrence of tetraploid shoots following grafting or decapitating of tomato plants. The chromosomes of our tetraploid seedling behave somewhat differently from those of their tetraploids. Winkler (9) found that reduction in his tetraploid was outstandingly normal, except that in "gigas" the chromosomes tended to clump together and countable first-metaphase groups with 24 pairs were rare. Jørgensen (4) reports finding one or two tetra-valents preceding the first metaphase, but states that 24 pairs may usually be made out easily. Like Winkler, he found the homeotypic

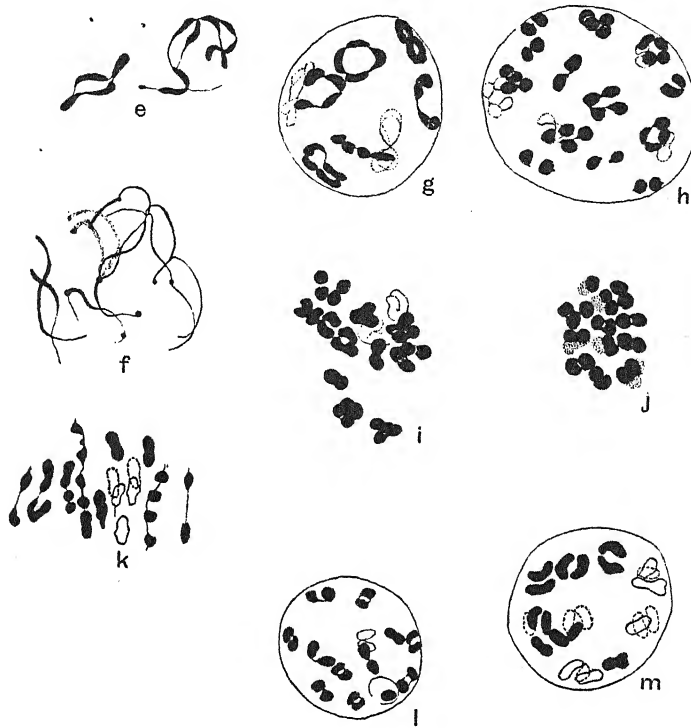
¹ M. M. Lesley ((7), p. 573), stated that the fact that the roots in chimeras were not distorted shows that tetraploid and diploid cells must have multiplied at very nearly the same rate. This statement is obviously erroneous. Since the tetraploid cells are about twice the size of the diploid, the former divide at approximately one-half the rate of the latter.

division very regular and with "very rare" exceptions both second metaphase plates contained 24 chromosomes. The exceptional second-metaphase plates had chromosome numbers ranging from 20 to 27. In our tetraploid seedling most of the chromosomes are in tetravalent groups and in no case have 24 pairs been observed at first metaphase. No diakinesis or first-metaphase has been seen with less than 7 tetravalents (Text-fig. 1, *h, i, j*), and 12 have been observed rarely at first metaphase. The sum of the chromosomes at this stage was never more than 48. Bivalents and univalents also occur at this stage. Lagging chromosomes are sometimes seen at first anaphase and, because of the presence of tetravalents, bivalents and occasional univalents, lateral first-metaphase and first-anaphase figures lack the uniformity of appearance characteristic of these stages in the diploid (Text-fig. 1, *k*). In Jørgensen's tetraploid "the plates had a very normal appearance at first metaphase." Of 49 single chromosome counts at second-metaphase in our tetraploid, 35 had 24, 3 had 22, 5 had 23, and 6 had 25; thus about 29 per cent. of the gametes derived from the tetraploid would be expected to have either more or less than 24 chromosomes. These observations on the maturation division accord closely with those of Belling(2) on the tetraploid *Datura*, in which most of the chromosomes are in tetrasomic groups (*ibid.* p. 188) and "a little over one-quarter of the pollen mother cells show uneven distribution of chromosomes." Of 122 lateral second-anaphase figures in our material, 99 had no laggards on either spindle and were very regular in appearance, 14 had one lagging chromosome assorting in each spindle, 4 had one dividing in each spindle, 1 showed one laggard dividing in one spindle and none in the other, and 4 had a few chromosomes free in the cytoplasm. Of 544 groups of microspores (tetrads) 474 were normal in appearance, while 55 had one and 15 had two microcytes in addition to four large cells. Few micronuclei were observed in the microspores. A diploid sib of our tetraploid had about 8 per cent. (74 in 891) of empty pollen grains, the rest appearing normal and about alike in size and staining capacity. Pollen of the tetraploid fixed at the same time varied considerably in both size and staining ability. Some grains were empty, others stained a very faint pink or light red and others were as deep red as the normal diploid pollen; about 30 per cent. (264 in 887) were obviously defective in some respect. Jørgensen's tetraploid had 75 per cent. good pollen as compared with 95 per cent. in his diploid.

Although the early prophase of the tetraploid seedling was not studied in detail, a few figures were observed and drawn (Text-fig. 1, *e, f*). The

threads are paired in the spireme in our tomato as in Jørgensen's⁽⁴⁾ Fig. 11 *a*.

The cause of the difference in tendency toward quadrivalent forma-



Text-fig. 1. Chromosomes in pollen mother-cells stained by Belling's iron aceto-carmin method. (e)-(k), tetraploid seedling 27,048,10, ($\times 3070$); (l), (m), triplo-C ($2n+1$) ($\times 2430$).

- (e) Typical appearance of chromosomes just preceding diakinesis in tetraploid.
- (f) Paired threads in early prophase of tetraploid.
- (g) Tetra-valents in early diakinesis in tetraploid.
- (h) Diakinesis with seven tetra-valents in tetraploid.
- (i) Eleven tetra-valents and two bi-valents just before first metaphase in tetraploid.
- (j) First metaphase group of tetraploid with eleven tetra-valents.
- (k) Lateral view of early first anaphase in tetraploid showing bivalents and tetra-valents dividing. The irregular appearance is characteristic.
- (l) Normal nucleus in diakinesis in simple trisomic (triplo-C) for comparison with (m).
- (m) Tetrasome formation in a $4n+2$ pollen mother-cell in simple trisomic triplo-C.

tion observed in Jørgensen's and our tetraploid tomatoes seems to deserve further study. Our tetraploid originated within a nearly pure line of Dwarf Aristocrat. Tetraploid pollen mother cells observed in a trisomic plant of this variety also showed a large amount of quadrivalent

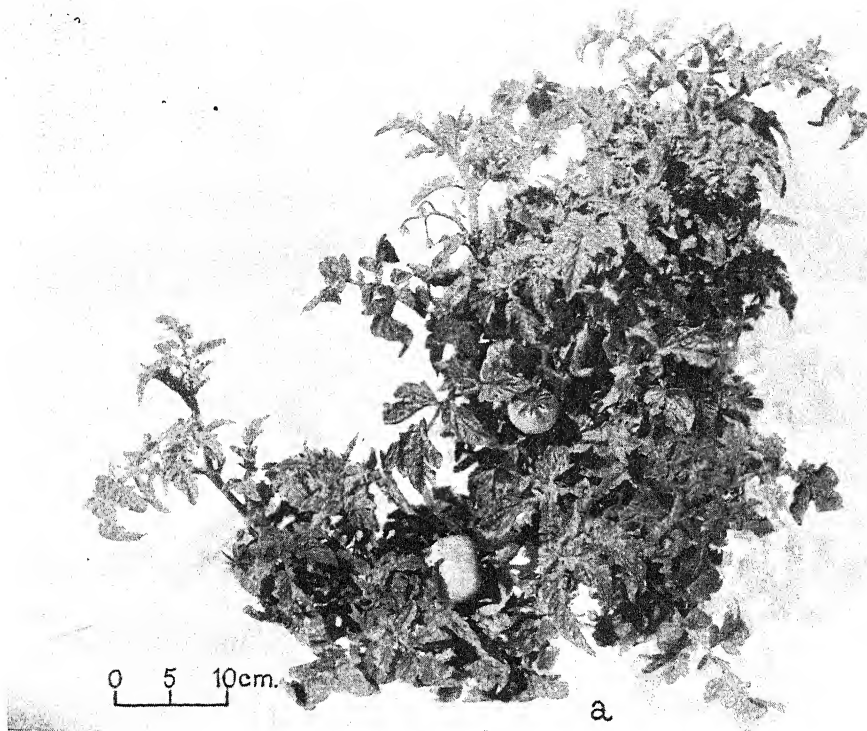
formation (Text-fig. 1, *m*). It is not stated whether the tetraploid plants which Jørgensen studied cytologically were hybrid or non-hybrid. It seems possible that the greater affinity of the chromosomes of the same set for each other may be a varietal characteristic. It is desirable that both tetraploids be grown and examined cytologically under the same conditions.

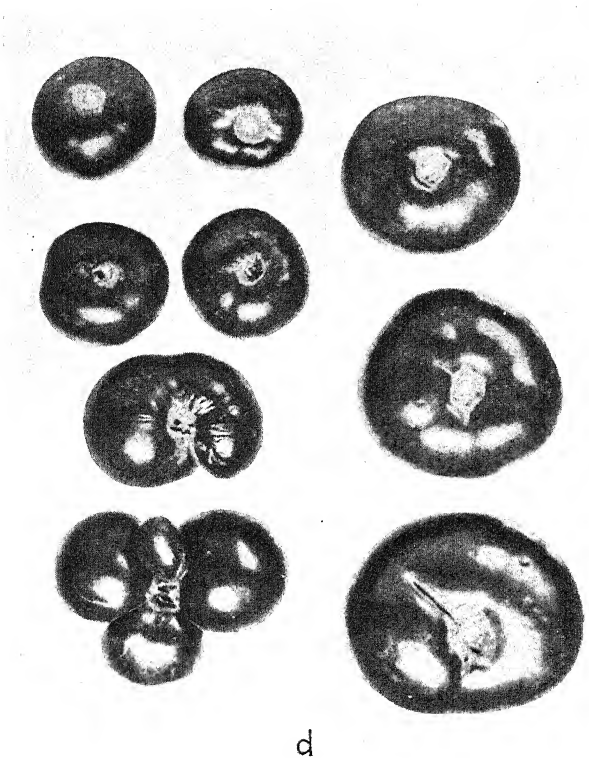
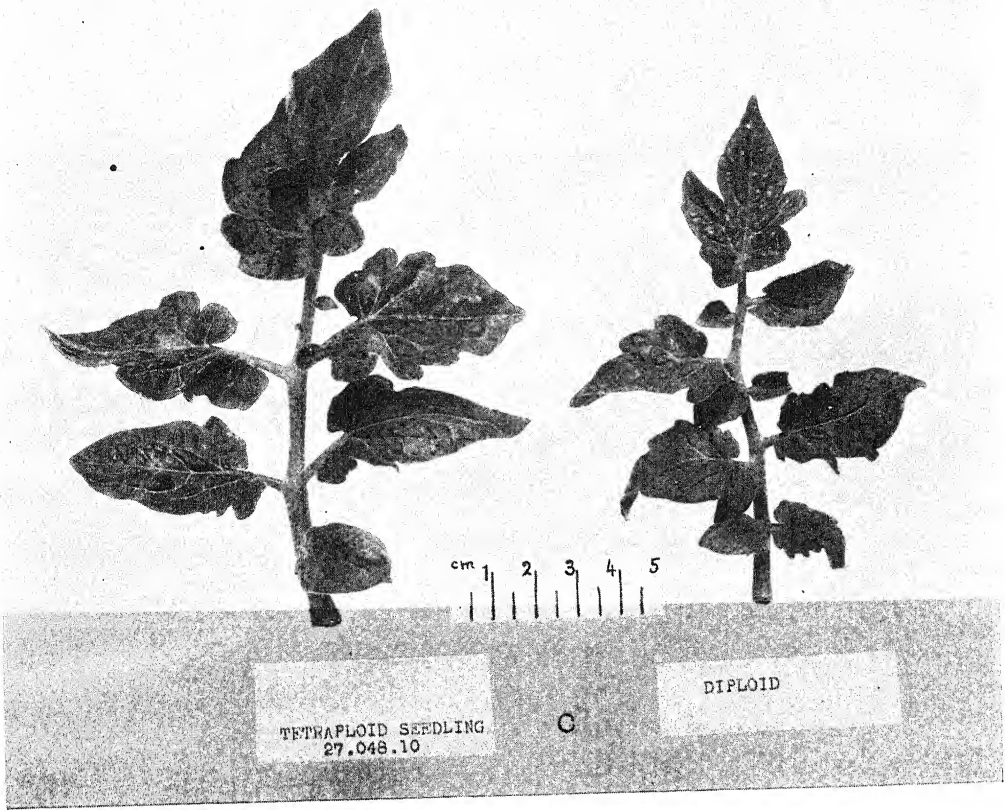
SUMMARY.

A tetraploid seedling tomato appeared in the F_1 of a cross between a double trisomic and a diploid ($2n = 24$). It has 48 chromosomes in its pollen mother cells. In gross morphology it resembles Winkler's (9) tetraploid bud-variant. No significant difference was found in the size of the plastids of tetraploid and diploid. Tetraploid selfed set viable seed sparingly. The cross tetraploid \times diploid seldom succeeds, but two F_1 plants were obtained. The reciprocal cross failed. The slower maturation of the megaspores and slower growth of the microspores of the tetraploid may account for the difficulty in making the crosses. Triploids occurred more commonly in the progeny of trisomic than of diploid parents. They evidently originate from the union of diploid and haploid gametes. Tetraploid pollen mother cells and, in simple- and double-trisomic plants, non-reduction of normal pollen mother cells have been observed. One triploid plant originated from a diploid gamete formed by reduction of a tetraploid mother cell. On account of the loss of the extra chromosomes of the double-trisomic parent, it is probable that the tetraploid seedling originated from doubling the chromosome number after fertilisation. At diakinesis and first-metaphase 7 to 12 quadrivalents were present in the pollen mother cells of the tetraploid seedling as compared with 1 to 2 quadrivalents observed by Jørgensen, and outstandingly normal reduction observed by Winkler in the tetraploid bud variants. Counts of second-metaphase plates suggest that unequal chromosome distribution is more common in the seedling than in the bud-variant tetraploids.

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EXPLANATION OF PLATES.

PLATE XVIII.

- a. Diploid Dwarf Aristocrat.
- b. Tetraploid seedling, 27,048,10, of Dwarf Aristocrat.

PLATE XIX.

- c. Leaf of diploid (right) and tetraploid (left).
- d. Six fruits of tetraploid (left) and three fruits of diploid (right).